University of Alberta

Probiotic therapy in critically ill enterally fed patients

By

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List of Symbols and Abbreviations

ANOVA	analysis of variance analysis
APACHE	acute physiology and chronic health evaluation
BMI	body mass index
CRP	C-reactive protein
EN	enteral nutrition
FOS	fructooligosaccharide
FiO_2	fractional inspired oxygen
ICU	intensive care unit
IgA	immunoglobulin A
IgG	immunoglobulin G
LME	linear mixed effects
LMR	lactulose mannitol ratio
LnLMR	log of lactulose mannitol ratio
LOS	length of stay
MODS	multiple organ dysfunction syndrome
RQ	respiratory quotient
SGA	subjective global assessment
VCO_2	carbon dioxide production per minute
VO_2	oxygen consumption per minute

Chapter One

Introduction

A. Rationale

The gut can not only be considered the organ of digestion and absorption, but also a major organ of the immune response. Approximately 70% of the immune system is localized in the gastrointestinal tract (Bengmark, 2002). Stimulation of the gut directly affects the defence mechanisms of the host. In healthy individuals, a diet enriched with fruits, vegetables, and grains supports viability of the bacteria within the gut (Bengmark, 2002). The host environment is non-hostile to these bacteria. However, once a patient is admitted to the intensive care unit (ICU), the administration of antibiotics coupled with difficulties providing enteral nutrition, disrupts this harmony. The normal, harmless bacteria begin to rapidly change, attaching to the gut wall more tightly in order to survive, and the harmless bacteria become more virulent. Pathogens become active and the normal gut flora prepares to defend itself against the virulent bacteria, together with the gut cells of the host. The gut becomes more permeable, leading to translocation of bacteria across the gut and subsequent sepsis. The role of the gut and its interaction with the bacterial flora is an area of clinical nutrition attracting new attention. Well-controlled clinical trials are required to establish efficacious usage of probiotic therapy in the critically ill.

Multiple organ dysfunction syndrome (MODS) has emerged in recent years as the major cause of death in adult ICU patients (Doig et al, 1998). This clinical condition is characterized by a state of hypermetabolism, which leads to a rapid

consumption of endogenous stores of protein and energy, immunological dysfunction and deterioration of organ function (Cerra, 1987). These physiological changes are orchestrated by a series of neuroendocrine events and the release of cytokines and mediators. Recent evidence supports a central role for the gastrointestinal tract in the triggering and sustaining of MODS (Deitch, 1992). Changes in gastrointestinal structure and function that lead to a loss of intestinal barrier function can result in an increase in bacterial translocation and absorption of toxins. Enhanced bacterial translocation then acts as a constant stimulus triggering the wide spread activation of pro-inflammatory cells and the release of other mediators of the metabolic response to sepsis.

The lumen of the intestine contains bacteria, bacterial products, and dietary antigens capable of initiating and sustaining inflammation. Indeed, the lumen of the gut is colonised by aerobic and anaerobic microflora with numbers reaching 10^{11} cfu/gm in the colon (Bengmark, 2002). Under normal conditions, bacteria remain within the lumen of the bowel, where they have important functions in metabolic and nutritional homeostasis. However, in disease states when the mucosal barrier is compromised, these microorganisms and their toxic products may enter the systemic circulation. Increased intestinal permeability and subsequent enhanced transmural migration of enteric bacteria to extra-intestinal sites has been demonstrated to occur where the intestinal mucosa is damaged by inflammation, infection, neoplasia, or trauma (Rowlands et al, 1999). Factors that predispose to the development of increased intestinal permeability are changes in the luminal micro-environment, ischemia, and malnutrition. Thus, treatment aimed at supporting the gut mucosal

barrier should have a beneficial effect in the prevention of MODS. These treatments would include maintaining adequate mucosal perfusion to optimize oxygen delivery, prevention of gastrointestinal haemorrhage, and, lastly, maintenance of protective luminal microflora using probiotic bacteria.

Probiotic bacterial strains, such as *Lactobacillus* and *Bifidobacterium*, are found naturally in the gut and have an important role in maintaining the gut barrier and protecting the host against pathogenic bacterial invasion. Several mechanisms have been proposed by which probiotics may exert their beneficial activity. These include: competitive exclusion of bacterial adherence and/or translocation (Marteau et al, 2001); release of bacteriocins and lactic acid which can inhibit growth of pathogens (Ahn and Stiles, 1990); production of butyric acid which enhances the turnover of enterocytes (Okamoto et al, 2000); probiotic-enhancement of barrier function by stimulation of mucus and sIgA production (Isolauri et al, 1993); an enhancement of macromolecular degradation by the gut mucosa (Pessi et al, 1998); a suppression of immune cell proliferation (Isolauri et al, 2001); secretion of a soluble factor which acts directly on epithelial cells to enhance barrier function (Madsen et al, 2001); and reduction of the number of mucosal gram negative bacteria by replacement with non-invasive, non-pathogenic probiotics (Madsen et al, 1999). Evidence to support probiotic use comes from studies showing that gut barrier dysfunction improves when the intestinal pool of gram-negative bacteria is reduced by the administration of selective antibiotics; lactulose is used to promote the growth of endogenous probiotic bacteria; or adsorbents are used to bind intraluminal endotoxins (Bengmark, 2002). Thus, restoring gut barrier function may be enhanced by multi-targeted therapy aimed

at reducing luminal aggressive microflora and reducing the systemic inflammatory response with steroids or anti-cytokine therapy. Indeed, the use of enteral diets that support probiotic bacteria has been advocated as an important new development in the support of enterally fed patients (Bengmark, 1996). These mechanistic features of probiotics make them ideal candidates to prophylactically treat MODS in the ICU.

B. Purpose

The purpose of the current research was to investigate the effects of probiotic therapy, VSL#3, on the development of MODS in critically ill, enterally fed patients. A double-blind randomised control trial was implemented. Adult patients from the Royal Alexandra Hospital ICU, Edmonton, Alberta were recruited for the study. Indices of intestinal permeability, nutritional intake, enteral nutrition tolerance, immune and inflammatory responses, and development of MODS were assessed.

Probiotic therapy has been studied in other areas of medical research. Benefits have been shown when used in the treatment of infantile diarrhea, ulcerative colitis and pouchitis, and prevention of post-operative recurrence of Crohn's disease (Madsen, 2001). The role of probiotic therapy for treatment of antibiotic-induced diarrhea, common in the intensive care unit, is less clearly documented. A paucity of research exists on the effects of probiotics in critically ill patients. This study provides insight into the effects of viable and sonicated probiotics on immune response, inflammatory response and progression of organ failure in ICU patients. The information will be useful in the evaluation of the therapeutic potential of probiotics in the critical care unit.

C. Hypotheses

1. Critically ill enterally fed patients will demonstrate reduced intestinal permeability, compared to control patients, when supplemented with a viable or sonicated probiotic compound.
2. Subsequently, reduced intestinal permeability, compared to control patients, will lead to reduced incidence of multiple organ dysfunction.
3. Critically ill, enterally fed patients will demonstrate reduced rates of diarrhea, compared to control patients, when supplemented with a viable or sonicated probiotic compound.
4. Critically ill, enterally fed patients will demonstrate a reduced inflammatory response, compared to control patients, when supplemented with a viable or sonicated probiotic compound.

D. Objectives

1. To assess the efficacy of probiotic-supplemented enteral nutrition on intestinal permeability and development of multiple organ dysfunction in critically ill patients.
2. To measure inflammatory and immune response, and enteral nutrition tolerance with and without probiotic therapy.
3. To assess incidence of diarrhea in enterally fed ICU patients with probiotic therapy.

Chapter Two

Literature Review

A. Introduction

Probiotic means *for life* in Greek. The term was originally created to contrast with antibiotic, which is a substance produced by one microbe to counter another. The definition of probiotic has been redefined as "a preparation of or a product containing viable, defined microorganisms in sufficient numbers which, by implantation or colonization, alter the microflora in a compartment of the host and by that exert beneficial health effects in the host" (Schrezenmeir and de Vrese, 2001). Simply, probiotics are living microorganisms that can affect the host in a beneficial manner. Prebiotics are nondigestible food ingredients that stimulate the growth and activity of probiotic bacteria already established in the colon. This literature review will focus on the effects of probiotics in the clinical setting and provide the background rationale for their application in the ICU.

B. Historical Background

Elie Metchnikoff, a Russian immunologist and Nobel prize winner, is often acknowledged as one of the first champions of probiotics. He suggested that the longevity of Bulgarians could be credited to their frequent consumption of sour milk, which contains lactobacilli (Lin, 2003). His work suggested that probiotics displaced the toxin-producing bacteria responsible for disease (Metchnikoff, 1908). In the past, methods of food preservation involved either the natural fermentation or drying of foods; thus, the human diet once contained several thousand times more bacteria than it does today. Changes in hygiene and

nutrition have altered the human gut microflora (Isolauri, 2001). Scientific work in the past decade supports the concept that there are clinical benefits to ingesting specific non-pathogenic organisms (probiotics).

C. Gut-Barrier Function

The intestine is a complex living system that participates in the protection of the host through a strong defence against aggressions from the external environment. This important defensive task of the intestine is based on 3 essential constituents: the microflora, the mucosal barrier, and the local immune system known as the gut-associated lymphoid tissue (GALT) (Bourlioux et al, 2003).

1. Microflora

The microflora in the human body consists of over 400 different species of bacteria, which generate metabolic activity, mainly in the colon. In healthy persons, the bacterial count of the colon reaches $10^{11} - 10^{12}$ colony forming units (cfu)/g. Although gram-negative anaerobes predominate in the distal ileum and colon, the composition of a human's microflora is unique, dependent on a vast number of factors. Many of these are host related, including age, race, gastric acid secretion and the presence of bile salts (Matarese et al, 2003). At birth, the gastrointestinal tract is sterile. During vaginal delivery, the infant's gut is colonized with microflora from the maternal birth canal. The flora of breastfed infants is quickly dominated by bifidobacteria, in contrast with that of infants fed formula milk. In breastfed infants, the flora also includes far fewer species liable to be pathogenic (Bourlioux et al, 2003). The composition of the flora evolves over time until it resembles the flora of adults. The species that exist in the

greatest quantities in the human intestine include *Bacteroides*, *Peptostreptococcus*, *Bifidobacterium*, *Eubacterium* and *Fusobacterium* (Salminen et al, 1998). The strains with health promoting properties include *Bifidobacterium* and *Lactobacillus*. Environmental factors that affect gut microflora include diet, stress, medication, and illness (Bengmark, 2002). Commensal flora are reduced early in the disease process. In infectious and inflammatory conditions the balance of the gut microecology is altered in such a way that the number of potentially pathogenic bacteria grows and the healthy interaction between the host and microbe is disturbed (Isolauri, 2001). In addition, antibiotic usage alters the protective gut flora resulting in potential overgrowth by pathogens, invasion and translocation of toxins, and life-threatening infections (Levy, 2000). Use of antibiotics promotes the emergence of resistant organisms, and multiple-antibiotic resistance has become a major public health issue (Levy, 2000).

The composition of the diet is also a factor contributing to the composition of the gut microflora. A diet high in fibre will provide substrate for the bacteria, producing short chain fatty acids, giving rise to acetic, propionic, and butyric acids. These acids affect colonic metabolism, the hepatic regulation of lipids and sugars, and the supply of energy to cells (Bourlioux et al, 2003).

2. Mucosal Barrier

The mucosal barrier is a complex physicochemical structure that separates the tissues from the luminal environment. Physically, the barrier consists of cellular components such as epithelial cell lining, the mucous layer (a gel formed by the

interaction of various mucosal secretions including mucins and surfactant phospholipids) (DeWitt and Kudsk, 1999). The first line of host defense is directed toward the exclusion of antigens, the elimination of foreign antigens that have penetrated the mucosa, and the regulation of ensuing antigen-specific immune responses (Isolauri, 2001). Intestinal permeability is a reflection of the gut-barrier function. Mucosal dysfunction may lead to increased intestinal permeability and aberrant antigen transfer and immune responses (Isolauri, 2001).

Mucosal barrier function ultimately depends on the physical integrity of the mucosa. The gastrointestinal mucosa has a surface area of approximately 300 to 400m^2 due to the microvilli that greatly amplify the surface area. The epithelial cells, and the enterocytes make up the majority of the mucosal epithelium (DeWitt and Kudsk, 1999). The enterocytes line both the crypts and the villi of the gastrointestinal mucosa. These junctions separate the external from the internal environment, acting as a selective barrier, attaching adjacent cells to one another.

3. GALT

The cells lining the intestinal epithelium can produce neuropeptides which also have an effect on barrier function by increasing the production of mucin and IgA, and stimulating GALT function. The GALT comprises a large body of lymphoid tissue that lines the mucosal surfaces of the body. The gastrointestinal tract contains approximately 70 to 80% of all immunoglobulin-producing cells. GALT contains activated B cells which ultimately serve to protect the mucosal immune system, producing secretory IgA. One function of SIgA is to prevent the

adherence of bacteria and viruses to the mucosal epithelium to defend against systemic invasion (DeWitt and Kudsk, 1999). IgA also increases mucin production. An underdeveloped GALT in animals devoid of an intestinal flora results in persistent enteritis, severe infections and poor survival. In addition, a metabolically active intestinal flora is critical for the maintenance of a healthy gut epithelium, vitamin production, bile acid metabolism and enterohepatic circulation (Saavedra and Tschernia, 2002). Additionally changes in the human microflora have been correlated with modulated local and systemic immune responses of the cells in GALT (De Simone et al, 1992).

D. Clinical Applications of Probiotics

1. Diarrhea

The best documented clinical application of probiotics is in the treatment of acute pediatric diarrheal disease. Rotaviruses continue to be a significant cause of infant morbidity and mortality, especially in developing countries (Majamaa et al, 1995). Isolauri et al have shown that *Lactobacillus rhamnosus* strain *GG* (LGG) provided significantly superior reductions in the duration of rotaviral diarrhea compared with standard pasteurized yogurt or placebo in pediatric populations in Finland (Isolauri et al, 1991). In her study, the duration of diarrhea was shortened from 2.4 to 1.4 days in patients who received *Lactobacillus* GG.

A larger European multi-centre trial in children 1 month to 3 years of age was recently reported. One group of 140 children randomly received oral rehydration therapy with placebo; the second group of 147 children randomly received oral rehydration therapy with 10^{10} cfu of *Lactobacillus* GG. In the rotavirus-positive

children in the lactobacillus-treated group, the diarrhea lasted 56.2 ± 16.9 hours vs 76.6± 41.6 hours in the control group (p=0.008) (Guandalini et al, 2000).

Infectious diarrhea has also been successfully treated by adding a combination of *Bifidobacteria* and *Streptococcus thermophilus* to powdered formula. Using the combination of cultures, a study was conducted with 55 infants of similar ages and weights who were inpatients at an American chronic care hospital over 17 months (Saavedra et al, 1994). The infants were fed either supplemented formula or control. The cumulative incidence of diarrhea was significantly reduced in infants receiving the supplemented formula (6.9% versus 31% of the control group). There was no difference in severity of diarrhea between the two groups when it did occur.

The clinical benefit of probiotics has been shown when used to treat conditions in which the gut microecology is disturbed by changes in the environment or by oral antimicrobial therapy. The prevention of traveler's diarrhea has been a popular target for probiotic trials, although results have been variable. *Lactobacillus* GG was found to be effective in the prevention of traveler's diarrhea in some studies but the effect may not be consistent depending on the geographic area or populations studied (Hilton et al, 1997; Oksanen et al, 1990). Other lactobacilli preparations have not produced any significant effects. As well, the many variables including the variety of probiotic agents studied, the lack of consistent documentation of diarrhea and the difficulties measuring compliance in these trials make results difficult to interpret.

Trials to prevent antibiotic-associated diarrhea also offer conflicting evidence (Cresci, 2001). Antibiotics can severely disrupt gut microbial ecology. Antibiotic-associated diarrhea (AAD) is the most common adverse side-effect of antibiotic therapy, occurring in 5-39% of patients (McFarland, 1998). Two major forms of AAD have been identified. One form does not identify a pathogen; typical clinical features include onset during antibiotic exposure, stool frequency that is dose-related, resolution upon discontinuation of the implicated antibiotic, and absence of inflammation (Beniwal et al, 2003). AAD can potentially be caused by microbial imbalance leading to a decreased level of endogenous flora, and decreased production of short-chain fatty acids (SCFA) in the intestine. SCFA are generated by the bacterial metabolism of complex carbohydrates (Levy, 2000). AAD can increase length of hospital stay, increase risk for other infections, decrease quality of life for patients, and increase the workload of nursing personnel. The second type of AAD is Clostridium-difficile-associated diarrhea. Symptoms of Clostridium-difficile diarrhea may persist for months, and may cause severe colitis, with the most characteristic lesion being pseudomembranous colitis. Clostridium difficile is the leading cause of nosocomially acquired intestinal infection in the United States (Pochapin, 2000). An average of 20% of patients who are initially infected with Clostridium difficile infection will suffer from recurrent disease after standard antibiotic therapy with either vancomycin or metronidazole (Pochapin, 2000). Scientists believe that the protective intestinal microflora is damaged by antibiotic treatment, and overgrowth or colonization by resistant organisms can occur when the flora is

suppressed by antibiotics (Levy, 2000). A recently published meta-analysis on probiotics in prevention of antibiotic associated diarrhea suggests that probiotics can be used to prevent AAD and that Sac*charomyces boulardii* and lactobacilli have the potential to be used in this situation (D'Souza et al, 2002; McFarland et al, 1995; Surawicz et al, 1989). The meta-analysis reviewed nine trials where probiotics were given in combination with antibiotics; the control groups received placebo and antibiotics. The combined odds ratio was 0.37 in favour of active treatment over placebo. The authors suggest that the efficacy of probiotics in treating antibiotic associated diarrhea is unproven, and a larger trial is needed in which cost of routine usage of probiotics is examined. Most trials looking at probiotic usage for AAD use a lyophilized form of probiotics. A recent trial was done to determine if commercial yogurt containing 10^6 cultures/g of *L. acidophilus*, *L. bulgaricus*, and *S. thermophilus* had any effect on hospitalized patients initiated on antibiotics (Beniwal et al, 2003). The intervention group received 227 g of vanilla flavoured yogurt twice daily. Patients receiving yogurt reported less frequent diarrhea (12% vs 24%; p=0.04), and significantly less total diarrheal days (23 vs 60). The authors concluded that dietary supplementation with an active-culture yogurt is a simple, effective, and safe treatment that decreases the incidence and duration of AAD.

2. Inflammatory Bowel Disease

The etiology and pathogenesis of inflammatory bowel disease (IBD), which comprises Crohn's disease and ulcerative colitis, remains elusive (Matarese et al, 2003; Schultz and Sartor, 2000). Genetic factors as well as environmental

triggers seem to play a role in the development and perpetuation of IBD. Among the environmental triggers, bacterial and viral organisms have been studied most frequently. It has been demonstrated that the concentrations of endogenous *Lactobacillus* and *Bifidobacteria* are significantly reduced in patients with active Crohn's disease, ulcerative colitis, and pouchitis (Sartor, 1999). These observations have stimulated interest in investigating various probiotic bacteria in treatment of IBD (Madsen, 2001). Several randomized controlled studies have evaluated the use of probiotics in preventing recurrence of Crohn's disease.

In a preliminary study, 15 patients with ulcerative colitis were treated with the probiotic preparation, VSL#3 (VSL Pharmaceuticals). In this study, 75% of the patients remained in remission after 12 months of probiotic therapy (Venturi et al, 1999). As a consequence of the benefits shown from the preliminary study, a double-blind, randomized trial was carried out to determine the efficacy of VSL#3 in the maintenance of chronic, relapsing pouchitis (Gionchetti et al, 2000). Many patients with ulcerative colitis need proctocolectomy with creation of an ileal pouch-anal anastomosis in order to preserve fecal continence. The most common complication to this surgery is pouchitis, an inflammatory process which can develop into refractory, chronic pouchitis. In this group of patients, Gionchetti found that 85% of patients treated with 6g/d of VSL#3 were asymptomatic, whereas 100% of patients in the placebo group had relapsed during the 9 month trial. Fecal concentrations of *Bifidobacterium, Lactobacillus and Streptococcus salivarius* increased during the period of probiotic administration, and then decreased one month following discontinuation of probiotics.

The efficacy of *S. boulardii* for the symptoms of Crohn's disease has also been studied. *S boulardii* or placebo, in combination with the standard treatment of mesalamine, was randomly given to 20 patients with active, moderate Crohn's disease for 7 weeks. There was a significant reduction in the frequency of bowel movements and in disease activity in the group receiving the *S. boulardii* (Plein and Hotz, 1993). Clinical relapses of Crohn's disease have also been shown to be significantly lower when patients are treated with 1g/d of *S. boulardii* with mesalamine vs standard treatment alone (Guslandi et al, 2000). These findings together indicate that probiotics could represent a form of maintenance therapy for patients with inflammatory bowel disease.

3. Colon Cancer

Currently, there is no direct evidence that probiotics can protect against the development of colon cancer, however, preliminary research on animal models is promising. Early studies demonstrated that colon tumerogenesis was reduced in rats given fermented milk, and DNA damage induced by various carcinogens was effectively prevented when animals were pretreated with various probiotics (Wollowski, 2001). In humans, consumption of lactobacillus and prebiotics has demonstrated a reduction of harmful bacterial enzymes and an increase in ß-glucosidase. An increase in ß-glucosidase could potentially be regarded as an advantage to health by releasing flavanoids with antimutagenic, anticarcinogenic, and immune-stimulatory effects (Wollowski et al, 2001).

4. Immune Function and Allergies

Probiotic bacteria have several potential immunomodulatory effects. Probiotics can help stabilize the gut microbial environment and the intestine's permeability barrier, and enhance systemic and mucosal IgA responses, thereby promoting the immunologic barrier of gut mucosa (Isoalauri, 2001). Probiotics exert positive effects on the immune system without eliciting harmful inflammatory responses. In healthy persons there is an immunostimulatory effect whereas in allergic persons down regulation of the inflammatory response was detected (Isolauri, 2001). Thus, the immunomodulatory effects of probiotic bacteria may depend on the immunologic status of the host. The exact role of normal gut microbiota in the development of allergy remains to be elucidated (Kalliomaki and Isolauri, 2003). Alterations in intestinal flora have been detected in infants suffering from allergic disease and those who later develop allergic disease. Delay in the compositional development of *Bifidobacterium* and *Lactobacillus* in gut microflora was a general finding in allergic children (Kalliomaki and Isolauri, 2003).

Perinatal administration of lactobacilli has been shown to decrease the development of atopic eczema during the first 2 years of an infant's life. In a double-blind study by Kalliomaki et al (2001), 132 subjects were enrolled. All subjects were considered high risk, meaning that all individuals had a relative with atopic eczema, allergic rhinitis or asthma. *Lactobacillus* GG at a dose of 10^{10}cfu/day was administered prenatally to mothers for 2-4 weeks prior to delivery, and postnatally to infants for 6 months. Breastfeeding mothers continued to take the product. At age two, there was a 50 percent reduction in

atopic eczema in the infants randomized to receive probiotics. The investigators hypothesized that probiotics may have promoted gut barrier function, decreased intestinal permeability, and increased anti-inflammatory cytokines (Kalliomaki et al, 2001). A follow-up study completed after the perinatal administration of probiotics showed that *Lactobacillus* GG persisted in preventing atopic eczema four years later (Kalliomaki et al, 2003).

Specific strains of the healthy gut microbiota have been shown to induce the production of IL-10 and transforming growth factor-ß, which possess an important regulatory role in the development of allergic type immune responses (Kalliomaki and Isolauri, 2003). Clinical testing has focused on immune function studies and not on actual incidence of disease. It is also difficult to extrapolate results from animal studies to humans. However, a New Zealand study measured immune changes in elderly subjects after supplementation with *Bifidobacterium* (Gill et al, 2001). Elderly subjects were chosen because the aging process has been shown to lead to a decline in adaptive immunity. Thirty healthy eldery volunteers participated in a 3-stage dietary supplementation trial with *Bifidobacterium lactis* HN019 lasting 9 weeks. During stage 1, subjects consumed low-fat milk, 200 ml twice daily for 3 weeks. During the intervention stage, they consumed milk supplemented with *B. lactis* HN019 in a dose of $5x10^{10}$ organisms/ d or a low dose of $5x10^{9}$ organisms/day for 3 weeks. During the final stage, the washout phase, they consumed low-fat milk. Increases in the proportions of CD4+ and CD25+ T lymphocytes and natural killer cells were noted after consumption of the probiotic. The greatest changes in immunity were

found in subjects who had poor pre-treatment immune responses. There was no statistical difference between the 2 doses of *B. lactis* on immune effects (Gill et al, 2001). Due to the short duration of most clinical studies, it is unclear whether any benefit to the immune system would be temporary or long-term. (Kopp-Hoolihan, 2001)

5. Other potential health effects

Modulation of gut flora with probiotics may also have an effect on urogenital health in women. Urinary and genital tract infections are often associated with colonic bacteria. Investigators have linked the consumption of probiotics to a reduced recurrence of Candida infection and bacterial vaginosis (Bruce and Reid, 2003). Lactobacilli has been shown to reduce the urogenital pathogen load and the risk of urinary tract and vaginal infections (Bruce and Reid, 2003).

Several prospective studies have been performed examining probiotic usage in the control of irritable bowel disease. *S. boulardii* and lactobacilli species have been tested in this group of patients. Some studies have shown that probiotics decrease pain, urgency, or bloating of irritable bowel (Nobaek et al, 2000). However, poor compliance to therapy is a factor making this group of patients a challenge to study (Kopp-Hoolihan, 2001).

There is also evidence to suggest that probiotics confer benefit to patients with lactose intolerance (Marteau et al, 1997). Lactobacilli produce lactase, which hydrolyzes the lactose in dairy products to galactose and glucose, thus preventing the intestinal distress that lactose maldigesters experience. *S. thermophilus*, L. *bulgaricus* and other lactobacilli used in fermented milk products deliver enough

bacterial lactase to the intestine and stomach where lactose is degraded to prevent symptoms in lactose-intolerant individuals (Marteau et al, 1997). Investigators have confirmed that lactose maldigesters can digest lactose in yogurt better than lactose in milk (Piaia et al, 2003).

E. Mechanism of Action

The methods by which probiotic bacteria exert effects in the host are not completely understood, although research is currently underway to understand their mechanism of action. There are many proposed mechanisms by which probiotics may protect the host from intestinal disorders. Much work remains attempting to classify the actions of individual organisms; in addition, the same probiotic may inhibit different pathogens by a different mechanism of action. One proposed mechanism that is receiving considerable attention is receptor competition, where probiotics compete with microbial pathogens for a limited number of receptors present on the surface epithelium (Marteau et al, 2001). Probiotics are also known to release antimicrobial compounds which inhibit both Gram positive and Gram negative bacteria (Ahn and Stiles, 1990). These inhibitory compounds may not only reduce the number of viable cells but also affect bacterial metabolism or toxin production (Mack et al, 1999). Probiotics may also increase levels of mucin secretion, which acts to block pathogen binding to epithelial receptors (Mack et al, 1999). Probiotics have been shown to enhance the activity of the intestinal immune system through the stimulation of macrophage and natural killer cells, and the increase of secretory immunoglobulin A, thus, immunoactivation is another area actively being studied. The underlying

mechanisms of immune stimulation are not well understood, however, it is suggested that selected strains of probiotics are able to alter mucosal and systemic immune function (Madsen, 2001).

F. Safety

Probiotics are normal commensal bacteria of human flora. There have been 143 human clinical trials between 1961 and 1999 using oral probiotic compounds, and no adverse effects or events were reported in any of the patients participating in these trials (Madsen, 2001; Naidu et al, 1999). However, individual patients may develop opportunistic infections to normal microflora. There are documented cases of systemic infections with probiotic ingestion; a recent review shows that most of these occurred in immunocompromised patients (Matarese et al, 2003). There were two reported cases of *L. rhamnosus* traced to possible, but not proven, probiotic consumption (Rautio et al, 1999; Mackay et al, 1999). One of these patients had a liver abscess and the other had endocarditis. Septicemia and endocarditis caused by *Lactobacillus* have been reported (Griffiths et al, 1992; Antony et al, 1996). These infections occurred in immunocompromised patients with aplasia (Chomorat and Esppinouse, 1991), organ transplantation (Patel et al, 1994), and human immunodeficiency virus infection (Schlegel et al, 1998). A recent review of Lactobacillus bacteremia was published out of Finland (Salminen et al, 2004). The authors reviewed 89 patients with *Lactobacillus* bacteremia. They were able to characterize the Lactobacillus species in 53% of the cases, revealing 25 patients were infected with *Lactobacillus rhamnosus* strains. In 11 of those cases, the strain was identical to the probiotic *L. rhamnosus* GG.

Predisposing factors to bacteremia were immunosuppression, prior prolonged hospitalization, and prior surgical interventions (Salminen et al, 2004).

With regard to ingestion of probiotics of yeast origin, there have been reports of fungemia caused by *Saccharomyces* species. Most of these cases are of immunocompromised patients receiving high doses of Ultra-Levure (Biocodex, Montrouge, France) containing 1.5g/d of *S boulardii* (Niault et al, 1999; Materese et al, 2003). Although these reports of infections are rare, use of probiotics in immunodeficient hosts should be done with caution.

Dosage of probiotic bacteria is an area of clinical uncertainty. Many studies show an effective dose of $10^9 - 10^{10}$ organisms per day on physiological effects such as diarrhea, lactose intolerance, and colon cancer. Effects of consuming lower levels has not been well documented, however research suggests that consumption of more than one strain of probiotic bacteria decreases the total probiotic requirement to demonstrate clinical benefits (Kopp-Hoolihan, 2001).

G. Viable vs Inactive Probiotics

A recent study by Rachmilewitz suggests the potential to use inactivated probiotics to confer health benefits to the host (Rachmilewitz et al, 2004). Previous studies have tried heat killing of probiotics to inactivate them, but this process destroyed the cellular structure and some of the beneficial aspects of viable probiotics. In this new study, the team used gamma radiation on the bacteria, minimizing metabolic activity. Next the team administered the irradiated probiotics to mice with experimentally induced colitis. The irradiated probiotics effectively ameliorated the colitis, similar to the viable probiotics given

to another group of mice with colitis, indicating that inactivated probiotics were as effective as live probiotics. The researchers indicated that the protective effects of probiotics were mediated by their own DNA rather than by their metabolites or ability to colonize the colon.

Heat-killed probiotics have been studied for their impact on acute and chronic diarrhea. Addition of a medication to the World Health Organization protocol for treatment of acute diarrhea in children was controversial (Simakochorn et al, 2000). The clinical efficacy of a probiotic (Lacteol Fort sachets; Laboratoire du L du Docteur Boucard, Houdan France) containing lyophilized heat-killed *Lactobacillus acidophilus* LB was assessed as an adjunct to oral rehydration therapy in children 3-24 months with acute diarrhea (Simakachorn et al, 2000). The children were randomized to received either 10 billion lyophilized heat-killed *L. acidophilus* LB or placebo at admission to hospital and at 12 hour intervals for a total of five doses. The researchers found that the addition of heat-killed *L. acidophilus* LB to oral rehydration therapy was effective in the treatment of acute diarrhea by decreasing the duration of diarrhea, and decreasing the number of rotavirus-positive children with watery stools.

The clinical efficacy of lyophilized heat-killed *Lactobacillus acidophilus* LB was compared with living lactobacilli in the treatment of chronic diarrhea in adults (Xiao et al, 2002). One hundred and thirty-seven patients with chronic diarrhea were randomly allocated to receive either a 4 week course of two capsules of Lacteol Fort twice daily or a 4 week course of a viable bacterial product, Lacidophilin. Frequency of stool was recorded, along with consistency,

abdominal pain, and distention. At the end of the treatment, the clinical symptoms were markedly improved in the Lacteol group, and bowel frequency was significantly lower in the Lacteol group, indicating that the heat-killed bacteria were more effective than living lactobacilli in the treatment of chronic diarrhea. Coconnier et al. showed that heat-killed *L. acidophilus*, strain LB, exhibited a high adhesive property in vitro. The ability of the heat-killed *L. acidophilus* to adhere to the brush border of the enterocytes, and to the mucus layer of the intestine inhibits the process of pathogenicity of a large variety of bacteria that cause diarrhea such as *Escherichia coli*, *Listeria* and *Salmonella* (Coconnier et al, 1993).

Recent in vitro experiments at the University of Alberta demonstrate the role of DNA from probiotic bacteria exerting anti-inflammatory actions on intestinal epithelial cells (Jijon et al, 2004). The investigators used both a freeze-dried culture of live probiotic bacteria, as well as purified DNA from probiotic strains to pretreat human colonic cells in culture. In both groups, cells subsequently exposed to pathogenic bacteria displayed significant reductions in activation of inflammatory pathway elements.

H. Probiotics, Prebiotics and Synbiotics

Probiotics have previously been defined as viable microbial food supplements which beneficially influence the health of the host (Schrezenmeir and De Vrese, 2001). Prebiotics are food ingredients that are largely not digested in the small bowel and can beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria (Schrezenmeir and De

Vrese, 2001). The only prebiotics which have been sufficiently analyzed in order to classify as functional food ingredients are the inulin-type fructans which include native inulin, enzymatically hydrolysed inulin or oligofructose, and synthetic fructooligosaccharides (Roberfroid, 2000). Inulin is produced industrially from chicory plants. Inulin and oligofructose are present naturally in wheat, onion, banana, garlic and leeks. The idea that inulin-type fructans are fermented by bacteria colonizing the large bowel is supported by in vitro and in vivo studies. In addition, the production of lactic and short-chain fatty acids as end products of the fermentation has been shown. In human in vivo studies this fermentation leads to the selective stimulation of growth of the bifidobacteria population (Gibson and Roberfroid, 1995). Other fibres available for use as potential prebiotic supplements in clinical nutrition include pectin, betaglucans, glucomannan, and algal fibres (Bengmark, 2002).

Evidence suggests that the addition of fermentable fibre to the diet alters the function and structure of the gut, and modifies the product of gut-derived hormones. Field et al (1999) found that the addition of fermentable fibres to the diet altered the function of GALT. They proposed three mechanisms underlying the immunomodulating effects of dietary fibres that change gut microflora. Firstly, direct contact of lactic acid bacteria or bacterial products with immune cells in the intestine may have an immunomodulating effect. Secondly, the gut microflora may modulate immune cells through the fermentation of dietary fibres to SCFA. It is well established that the fermentation of inulin and oligofructose increases the production of SCFA. In turn, SCFA lower the pH of the colon,

inhibiting pathogen growth and improving mineral absorption. Finally, there is some evidence that the addition of fermentable fibres to the diet can increase mucin production. Greater mucin production might contribute to the lower incidence of bacterial translocation in the animal model (Schley and Field, 2002).

Combining probiotics and prebiotics in what has been called a synbiotic could beneficially affect the host by improving survival, implantation and growth of probiotic bacteria in the gastrointestinal flora. A study in children with gastroenteritis showed a significant decrease in duration of diarrhea when randomized to receive a diet supplemented with a synbiotic (Ahmad et al, 2000). However, a recently published clinical study showed no measurable effect of synbiotics on gut barrier function in elective surgical patients (Anderson et al, 2004). Although not extensively studied, it is postulated that by improving the gastrointestinal tract's microbial balance, the effects of probiotic bacteria with prebiotics could be enhanced or even synergistic (Roberfroid, 2000).

I. Intestinal Permeability and MODS

The multiple organ dysfunction score is a validated, objective scale to measure the severity of multiple organ dysfunction as an outcome in critical illness (Marshall et al, 1995). The scale, calculated using physiologic measures of dysfunction in six organ systems, correlates strongly with risk of ICU and hospital mortality. Increased intestinal permeability has been shown to correlate with the development of MODS in critically ill ICU patients (Doig et al, 1998). This group of researchers demonstrated that intestinal permeability on admission to ICU was predictive of subsequent development of MODS. The observations in

this study led the authors to postulate that gastrointestinal dysfunction may be a stimulus for development of MODS in the critically ill population.

J. Application to the Intensive Care Unit

Few studies examining the role of probiotics in the ICU have been performed. A nursing student in Hong Kong conducted a small study examining the role of viable probiotics in the development of multiple organ dysfunction in a mixed ICU. Nineteen patients were randomized to receive viable *Lactobacillus plantarum* 299 while another 19 patients received heat-killed *Lactobacillus plantarum* 299 (controls); 5/19 (26%) died in the treated group versus 8/19 (42%) in the control group. Although the difference did not reach statistical significance, it prompted investigators to undertake a larger study, which is currently underway (Gomersall CM, 1998).

A recent study was undertaken at the University of Berlin examining the role of *Lactobacillus plantarum* 299 versus heat-killed *Lactobacillus plantarum* 299 in the incidence of post-surgical infections (Rayes et al, 2002). After major abdominal surgery, patients were randomized into one of three groups. The first group consisted of patients on parenteral nutrition or fibre-free enteral nutrition; patients randomized to the second group received fibre-containing enteral nutrition with living *Lactobacillus* at a daily dose of 10^{10} organisms versus the third group which received fibre-containing enteral nutrition with heat-killed *Lactobacillus* at a dose of 10^{10}organisms. The incidence of infections was significantly lower in the patients who received a fibre-supplemented formula with probiotics as compared to the group on TPN or EN with no fibre or

probiotics. There were no benefits of living *Lactobacillus* as opposed to heat-killed Lactobacillus in the entire study population, but benefits of viable *Lactobacillus* were observed in the patients with gastric and pancreatic resections. Unfortunately, the sample population was too small to show statistical significance (Rayes et al, 2002).

A randomized trial of probiotics and fibre was conducted in Hungary in patients with acute pancreatitis (Olah et al, 2002). Patients were randomized into two double-blind groups. The treatment group received a freeze-dried preparation containing live *Lactobacillus plantarum* 299 in a dose of 10^9 organisms, together with a substrate of oat fibre, for one week by nasojejunal tube. The control group received a similar preparation but the *Lactobacillus* was inactivated by heat. Forty-five patients completed the study. Twenty-two patients received treatment with live and 23 with heat-killed *Lactobacillus*. Infected pancreatic necrosis and abscesses occurred in one of 22 patients in the treatment group, compared with 7 of 23 in the heat-killed group (control) ($p=0.023$). The authors concluded that live *Lactobacillus plantarum* 299 at a dose of 10^9 organisms per day was effective in reducing pancreatic sepsis and the number of surgical interventions. Of secondary note, the single patient in the treatment group who developed infection had signs on day 15 after admission, eight days after probiotic treatment had been discontinued. To be protective, the authors suggest that treatment with probiotics and prebiotics should be considered for 2-3 weeks, or for as long as stool cultures show colonization with potential pathogens.

A larger study was conducted in the ICU setting using *Saccharomyces boulardii* in critically ill enterally-fed patients. A total of 128 patients were studied in eleven intensive care units. Sixty-four patients received *S. Boulardii* 50 mg four times a day, and 64 patients received placebo. Treatment with *S. boulardii* reduced the mean percentage of days with diarrhea from 18.9% to 14.2% ($p=0.0069$) (Bleichner et al, 1997).

The challenges to feeding ICU patients are many and varied: inability to feed successfully via the gastrointestinal tract causing gut atrophy, inhibition of important gastrointestinal secretions and the limited intake of fibre. Antibiotic therapy alters microflora balance allowing proliferation of pathogens and their toxic products. ICU patients acquire nosocomial infections at a much greater rate than patients elsewhere in the hospital (Bengmark, 2002). Positive effects of probiotics have been demonstrated in specific health conditions with specific products. However, unreasonable extrapolation of results to other clinical situations, products or doses is not warranted without further evidence (Marteau and Boutron-Ruault, 2002).

Chapter Three

Experimental Design and Methodology

A. Experimental Design

The probiotics trial was a randomized, double-blind prospective study design. Patients admitted to the adult ICU at Edmonton's Royal Alexandra Hospital were screened for eligibility to participate in the study. Patients were randomized to receive one of three treatments: live probiotic, probiotic sonicates, or placebo. All patients were initiated on an enteral feeding protocol using a formula containing a prebiotic compound, fructooligosaccharide (FOS). The subjects received the treatment for six consecutive days. Pre- and post blood collections were performed, along with daily urine collections for assessment of intestinal permeability. Physiological data were collected daily to determine severity of illness and development of MODS. Diarrheal scores were also measured daily. Ethical approval for the study was obtained from the Human Research Ethics Board, Biomedical Panel, of the Capital Health Authority, Edmonton. Operational approval to conduct clinical research at a Capital Health Authority facility was obtained from the clinical ICU director. The study investigators obtained informed consent from family members.

It was estimated that a sample size of 45 subjects was required containing 15 subjects per treatment group. Sample size was initially calculated based upon an α level of 0.05 and power of 90% using independent t-test calculations for intestinal permeability changes. Eligible subjects were males or females ≥18 years of age who could be fed enterally within 48 hours of ICU admission, and who were anticipated to require enteral nutrition for greater than

48 hours. Patients were excluded for the following reasons: 1) they could not be fed via the gastrointestinal tract 2) they presented with renal failure, pancreatitis or short gut syndrome 3) they presented with pre-existing sacral ulcers 4)they were HIV positive, or had a previous bone marrow, lung, or liver transplant 5) they were receiving mannitol or lactulose, or the medical team anticipated initiation of one of these drugs over the upcoming 7 days, or 6) they were not expected to survive 7 days given their preexisting uncorrectable medical condition. Family members of patients who met recruitment criteria were offered participation in the trial. Family members were provided with an information package explaining purpose of, and risks and benefits to the patient. They were given time to read the package, and to discuss any concerns or questions with the study researchers prior to giving consent or refusal (Appendix A).

B. Methodology

1. Treatment Regimes

Patients were randomly assigned to receive one of three treatments: a fibre-containing enteral formula Jevity ® Plus (Ross Nutritionals, Illinois, U.S.A.) with placebo, Jevity ® Plus supplemented with viable VSL#3 (CSL, Milan, Italy), or Jevity ® Plus supplemented with a bacterial sonicate of VSL#3. VSL#3 is a probiotic compound consisting of three species of *Bifidobacterium*, four strains of *Lactobacillus* and one strain of *Streptococcus*. Jevity ® Plus is a polymeric enteral formula containing 22g of fibre per 1000ml, which includes 10g of FOS per1000ml, and 12 g of a patented soluble and insoluble fibre blend. Enteral nutrition was provided to study patients within a maximum of 48 hours from the time of ICU admission. The pharmacy department was notified of enrolment into the probiotic trial and delivered a foil wrapped 30 ml syringe

containing probiotic or placebo to the patient's bedside twice daily at 0900h and 2100h, according to the randomization schedule in the Pharmacy department at the Royal Alexandra Hospital. The ICU bedside nurse gave the study compound via the feeding tube and then flushed the tube with 20 ml of sterile water. The live probiotic and placebo were prepared by on-site pharmacy staff and were administered to the patient within 60 minutes of reconstitution. The bacterial sonicates were prepared by the lab technician at the University of Alberta, Division of Gastroenterology laboratory (Appendix B). They were immediately frozen and then transported to the Royal Alexandra Pharmacy where they remained frozen until thawed for patient delivery. The bacterial sonicates were bolused to the patients within one hour of thawing. The patients randomized to receive probiotics were given 3 g of VSL#3 twice daily providing a total of 9×10^{11} bacteria. All treatments were diluted to a final volume of 30mls. Jevity ® Plus feeds were initiated and progressed by standard ICU protocol, already utilized in the Capital Health Region. Patients remained on the study for seven days. If the patient was able to discontinue enteral nutrition or was ready to be transferred out of the intensive care unit prior to 7 days, the study was discontinued early. The intestinal permeability measurements were performed daily until study termination, and the study compound was given twice daily until study termination.

2. Intestinal Permeability

Intestinal permeability was measured daily for 7 days using a standardized protocol developed by Doig et al (1998). The first measurement was performed on Day 1 prior to the patient receiving enteral nutrition, and the probiotic/placebo treatment. A syringe containing 7.5 mls of lactulose was prepared in pharmacy. The lactulose was

sent daily to the bedside nurse along with 2 g of mannitol. The mannitol was reconstituted with 20 ml of distilled water and administered daily during the ICU stay. Twenty ml of water was given to rinse the feeding tube after administration of the sugar solution. Feeding with enteral preparations was temporarily interrupted during administration of the sugar solution, but was immediately resumed following the rinse solution. The excreted portion of each sugar marker in urine was collected for 6 hours via a standard urinary catheter collecting system to which gentamicin was added. Urine collected was placed in a collection bottle containing 5 ml of 10% thymol. The urine was drained from the catheter bag every hour into the collection jug. The jug was kept on ice at the bedside for the 6 hour collection time. The collection bottle was refrigerated at 4°C and then mixed and measured for total volume, prior to taking 2-15 ml aliquots of urine. All samples were frozen to –70°C within 24 hours. The 15 ml aliquots of urine were sent in batches on dry ice to the University of Calgary, Department of Medicine. A duplicate sample was retained at the University of Alberta in the –70°C freezer. Measurement of the urinary concentration of sugars was made using standardized HPLC methodology (Appendix C). (Doig et al, 1998)

3. Hematological Analysis

Six ml of blood was collected by research staff on Day 1 and Day 7 of the study. The blood was sent to the hospital-based laboratory where it was allowed to clot and then centrifuged. C Reactive Protein (CRP), IgA and IgG were measured on Day 1 of the study to determine baseline levels prior to initiation of enteral nutrition and the study treatment. All subjects had blood sampling repeated at the completion of the study which was defined as Day 7 following the 6 hour urine collection for intestinal permeability. In

the cases where it was necessary that a subject complete the study prior to Day 7, the blood sample was taken at the conclusion of the last intestinal permeability collection. Analysis was performed by the staff at Dynacare-Kasper Laboratories (Edmonton, AB). The Beckman IMMAGE system was utilized, measuring the rate of increase in light scattered from particles suspended in solution as a result of complexes formed during an antibody-antigen reaction.

4. Nutritional Assessment

All subjects enrolled in this study were fed enterally with Jevity ® Plus. Enteral nutrition was initiated at 20ml/ hr and progressed by 25ml/hr every 4 hours until the target rate established by the dietitian was achieved. Energy requirements were initially calculated using the empiric formula 25-30 kcals/kg, and protein requirements were calculated using the formula 1.2-1.5 g/kg protein. Since body weights are not routinely performed by ICU nursing staff, weights recently documented in medical records were used in the calculations. In the absence of documented weights, family members were asked to estimate the patient's body weight. The weight was then recorded as an estimated weight. Heights were taken from medical records, or measured at the bedside. Daily energy and protein intake from enteral feeds were recorded. (Appendix D) Body mass index calculated by the formula "wt(kg)/ht(m)^2" and subjective global assessment (SGA) were assessed at initiation of enteral nutrition (Appendix E).

5. Indirect Calorimetry

Once patients had achieved their target rate of enteral feeding, an indirect calorimetry measurement was performed to confirm adequacy of enteral nutrition. Patients were assessed using a Sensormedics Deltatrac II indirect calorimeter (Sensormedics, Yorba

Linda, CA) for a minimum of 20 minutes. Patients did not receive any analgesia or stimulation or undergo any ventilatory changes for 30 minutes before the test or during the measurement. Acceptable variation in VO_2 and VCO_2 was defined as <15%, and acceptable variation in respiratory quotient (RQ) was defined as <10% (Porter and Cohen, 1996). Measurements that did not fit within these variability ranges were not used. Energy requirements were reassessed based upon the indirect calorimetry results, and enteral feeding rates were adjusted to meet resting energy expenditures.

6. Hart & Dobb Scale

Diarrheal episodes were measured daily using the Hart & Dobb diarrheal scale, assigning a numeric value to frequency, consistency and volume of fecal output (Appendix F). The bedside nurses were asked to record all of the patient's bowel movements on the forms provided by research staff, and rate them according to consistency and volume. Diarrhea was defined as a score of 12 or higher in a 24 hour period. (Hart and Dobb, 1988) The forms were double-checked against the patient's medical chart where bowel movements are routinely recorded.

7. Multiple Organ Dysfunction Syndrome Score (MODS)

MODS is a validated outcome measure developed for intensive care. (Marshall et al, 1995) It was developed using simple physiologic measures of dysfunction in six organ systems which correlate strongly with the ultimate risk of ICU and hospital mortality. An increase in MODS score reflects the development of organ dysfunction during a patient's ICU stay. The MODS score was calculated by research staff on day 1, 4 and 7 of the study (Appendix G). If the patient remained in intensive care, the score was calculated

weekly (Days 14, 21 and 28) until Day 28. If the patient did not complete the 7 days of treatment, MODS was calculated on the final day of the study.

The parameters used to calculate MODS for each individual system are as follows: (a) respiratory (PO_2/FiO_2), (b) renal (serum creatinine), (c) hepatic (bilirubin), (d) cardiovascular (pressure adjusted heart rate or PAR), (e) hematologic (platelets) and (f) neurologic (Glasgow Coma Scale). In the absence of central venous pressure measurement, the pressure adjusted heart rate was assumed to be normal and given a score of 0. When bilirubin measurements were not available on the date when MODS was scored, the bilirubin result from the nearest date was used.

On the days when MODS was scored, a number between 0 and 4 was assigned to each of the six mentioned parameters, with a score of 4 representing greatest dysfunction. A final score between 0 and 24 was calculated. Increasing values correlate closely with ICU and hospital mortality rate, and with increased length of ICU stay.

8. Apache II Scores

Acute Physiology and Chronic Health Evaluation II (APACHE II) scores were calculated on data during the 24 hours prior to initiation of enteral nutrition in order to determine the severity of illness of the enrolled patients. The score was based on ICU data only, and excluded data from emergency or other institutions. APACHE II scores give a measurement of severity of illness of ICU patients based upon age, previous medical history, recent elective or emergency surgery, and the presence of abnormally high or low ranges for twelve separate physiological variables (Appendix H). The determinants for the calculation of the APACHE II score were in accordance with previously published criteria. (Knaus et al, 1985)

9. Concurrent Antibiotic Usage

Antibiotic usage was recorded daily and coded according to the pharmaceutical classifications for antibiotics. The antibiotic groupings were as follows: penicillins, aminoglycosides, macrolides, quinolones, cephalosporins, antifungals, metronidazole, vancomycin, and clindamycin (Appendix I). Individuals treated with antibiotics have an increased susceptibility to new infections, sub optimal gastrointestinal secretions, and an altered gastrointestinal flora. (Bengmark, 2002) As well, many antibiotics have gastrointestinal side-effects including diarrhea.

10. Feeding Tube Type and Position

The type of feeding tube used was recorded daily, and the position of the tube was verified by chest x-ray to verify proper placement of feeding tubes, as well as to rule out any potential differences amongst treatment groups due to site of probiotic administration (stomach vs small bowel).

11. Demographic Data

Hospital and ICU admission date and time was recorded along with patient demographics: age, gender and diagnosis. Discharge date and time were recorded. If the patient died during their hospital stay, the etiology of death was obtained from the death certificate. When the subjects completed the study, the primary reason for ending study participation was recorded (Appendix J).

12. Data Validation

At the start of each nursing shift change (0700h and 1900h), the bed-side nurse was contacted by one of the study investigations to review expectations of the nurse's role in the study. A worksheet was left on the patient's chart to indicate the step-by-step

processes. A contact number for questions and concerns was provided on a 24 hour basis. The timing of the lactulose, mannitol, and study compound administration was logged on the computerized medication system, and validated by the research investigators. A form was provided to the bedside-nurse to record any interruptions to the tube feed, and the reason for the interruptions. These forms, along with the Hart & Dobb forms, were collected the following day by the research nurses.

C. Statistical Analysis

The data were analyzed using the statistical software program SPSS 12.0, Statistical Package for the Social Sciences, (SPSS Inc, Chicago, IL) and Splus 6.1 (Unix; Statistical Science Inc., Seattle, WA). Descriptive statistics including age, diagnosis, antibiotic usage, severity of illness were compared between the treatment groups and control group. ANOVA was performed on all baseline data. If the ANOVA was significant, post-hoc independent t-tests were done to compare groups. All outcome variables (development of multiple system organ disease, intestinal permeability, enteral nutrition intake, pre- and post IgG and IgA and C Reactive Protein levels) were assessed with a repeated measures ANOVA. All statistical tests were two-sided and were performed at the $p<0.05$ level of significance. Daily intestinal permeability measurements were assessed to determine probiotic treatment affects on intestinal permeability. Intestinal permeability measures were reported as the lactulose mannitol ratio (LMR). LMR results were converted to their natural log (ln) values to normalize the distribution for analysis. The abbreviation used for reporting purposes is ln(LMR). The upper limit of normal for the LMR was earlier defined by Doig et al (1998) as the mean +

3 SD of several hundred normal volunteers, and is 0.030. Therefore, an ln(LMR) of –3.50 represents the upper limit of normal for this study. Cohorts were compared for daily changes in permeability through the use of a linear mixed-effects (LME) model, a technique allowing comparisons between the means of cohorts, unit changes in permeability per unit change per day, inclusion of effects of daily changes of physiologic dysfunction, and accounting for individual variability between patients. (Doig et al, 1998)

Chapter Four

Results

A. Participant Characteristics

Two hundred and ninety two patients were screened for eligibility for the probiotics trial between January 1, 2003 and January 9, 2004. Twenty-eight of the patients screened were enrolled in the trial. Most common reasons for ineligibility for the study included enteral feeding had already commenced, greater than 48 hours had elapsed since ICU admission and decision to begin enteral nutrition, or no family were available to provide informed consent. At enrolment, there were no significant differences between groups in age, gender, severity of illness, or body mass index (BMI) (Table 1). During hospitalization there were no differences between groups in number of antibiotics received, or survival. Post-hoc analysis shows a significantly greater length of stay (LOS) in the group receiving the sonicates, but no significant difference for ICU LOS was shown.

B. Side Effects of Treatment

No adverse effects of placebo or probiotic therapy were noted at any time during the study. One patient was switched to total parenteral nutrition during the study due to a bowel obstruction. At the conclusion of the study, it was determined that he had received live probiotic therapy. One patient in each of the three treatment groups died during their ICU admission; five more patients expired on the ward following transfer out of the ICU, three of whom received probiotic sonicates, one received live probiotics, and one subject received placebo. Cause of death for patients who died in ICU included respiratory failure (1 patient on sonicates),

congestive heart failure (1 patient on live probiotics), and myocardial infarction (1 patient on placebo). Of the patients who expired later during their hospitalization, cause of death was respiratory failure (2 patients), cardio-pulmonary failure (1 patient), and intracranial haemorrhage (1 patient).

C. Nutritional Parameters

Upon enrolment into the study, the nutritional status of all subjects was assessed by subjective global assessment. Patients in the treatment groups were similar in terms of nutritional status with 18 of the 28 patients (64%) found to be well-nourished prior to ICU admission (Table 2). Energy and protein intake from Jevity Plus® feeds was calculated daily. Mean daily energy intake was compared to energy requirements derived from indirect calorimetry measurements as described in the methodology section. Mean protein intakes were compared to protein requirements calculated by formulaic methods. Post-hoc analysis shows a significantly greater percent of energy requirements achieved in the placebo group versus the live probiotic group ($p=0.034$; Table 2). No significant differences existed between treatment groups for mean energy and protein intake. The two most common reasons for interrupting enteral nutrition included temporarily stopping feeding for medical procedures, or stopping nutrition due to increased gastric residuals, deemed to be greater than 150mls by ICU enteral feeding protocol.

D. Hematological Parameters

Repeated measures analysis of variance procedures resulted in no significant interactions. Thus only the effects of time and treatment are reported.

1. Immunoglobulin G (IgG)

IgG was measured on Day 1 prior to probiotic or placebo treatment, and prior to initiation of enteral nutrition. IgG measurement was repeated at Day 7 or at study completion if enteral feeding was discontinued prior to seven days. Twenty-six patients had pre- and post-IgG measurements completed. One patient died during the first 7 days of treatment, and one patient was transferred out of the region on an emergent basis; thereby post IgG levels were not obtained on these two patients. The results indicate that there was a significant increase in IgG levels over the 7 day period ($p=0.026$), however, the increase in IgG levels was not significantly different between treatment groups ($p=0.367$; Table 3, Figure1). Figure 1 demonstrates that the largest increase over time occurred with the subjects who received live probiotics.

2. Immunoglobulin A (IgA)

Pre- and post-IgA levels were completed on 26 of the 28 patients. The results showed a significant increase in IgA levels over time ($p=0.021$) but no significant differences between treatment groups ($p=0.812$; Table 4). Figure 2 shows that the largest increase over time was demonstrated with patients who received live probiotics (1.94 to 2.61 g/l).

3. C-Reactive Protein (CRP)

CRP levels were measured on Day 1 and 7 on 26 of the 28 enrolled patients. There was a significant decline in CRP levels in all treatment groups ($p=0.003$) over time. There were no significant differences between treatment groups ($p=0.932$; Table 5, Figure 3). Figure 3 shows that the largest decreases over time occurred in the patients who received the sonicates and the placebo.

E. Incidence of Diarrhea

The incidence of diarrhea was determined by daily measurement of the Hart & Dobb Score described in the methodology. A patient was deemed to have diarrhea if the Hart & Dobb score was 12 or greater. The number of days the subjects had diarrhea was compared to the number of days the patient was on the study, and receiving probiotic or placebo therapy. The number of days with diarrhea was compared to the days fed. Results show that patients receiving placebo had a 22.7% incidence of diarrhea compared to 14.4% incidence of diarrhea in the patients receiving live probiotics, and 11.1% incidence of diarrhea in patients on probiotic sonicates. Results were not statistically significant ($p=0.447$ between all 3 treatment groups, and $p=0.222$ between placebo and sonicates; Table 6, Figure 4).

F. Multiple Organ Dysfunction Syndrome (MODS)

MODS scores were calculated on Days 1, 4, and 7. If patients remained in ICU, MODS scores were calculated weekly until Day 28. Since 10 of the 28 patients were discontinued from the study by day 7, MODS scores from Day 1 to Day 4 are shown ($n=26$). MODS scores from Day 1 to Day 4 decreased in the patients who received live probiotics, and increased in the patients who received placebo and sonicates. Results were not statistically significant amongst treatments ($p=0.930$; Table 7, Figure 5). Of the 18 patients who remained in the study until Day 7, MODS scores increased in the placebo group ($n=6$), decreased in the sonicate group ($n=7$), and increased in the live probiotic group with $n=5$. Results were not statistically significant amongst treatment groups ($p=0.243$).

G. Intestinal Permeability

Intestinal permeability was measured daily and reported as the lactulose/ mannitol ratio (LMR). As described by Doig et al, LMR results were converted to their natural log (ln) values to normalize the distribution for analysis (Doig et al, 1998). Converted results are reported as ln(LMR). An ln(LMR) of –3.50 represents the upper limit of normal for intestinal permeability results. Individual results of the treatment groups are plotted in Figures 6-8. In each treatment group, laboratory analysis of two Day 1 intestinal permeability measures was impossible due to interference of the carbohydrate in the enteral formula with the lactulose and mannitol probes. A lowess smoothing plot of patients in all treatment groups is shown in Figure 9. Smoothing plots of each treatment group are shown in Figures 9a-c. Lowess smoothing plots model changes in intestinal permeability over time. Intestinal permeability results, ln(LMR), showed no significant differences between the live probiotic group and the placebo group ($p=0.88$). A trend was observed such that decreased intestinal permeability was seen in subjects who received probiotic sonicates compared to the subjects who received placebo and live probiotics ($p=0.06$). The majority of patients in the sonicates treatment group had normal permeability at the start of the study. In contrast, patients in the live probiotics treatment group and placebo treatment group had higher intestinal permeability at study initiation. A significant decrease in intestinal permeability over time in all treatment groups collectively was determined ($p<0.003$), and is demonstrated graphically in Figure 9. Age and APACHE II scores had no relationship with intestinal permeability results (data not shown). There was a

positive relationship shown between energy intake and intestinal permeability; as energy intake increased, intestinal permeability also increased ($p=0.01$).

Table 1: Demographics and Clinical Variables of Study Participants by Treatment Group

	Live probiotics n=10	**Sonicates** n=9	**Placebo** n=9	**Significance**
Age (yr)	60.4±17.9[1]	66.6±18.9	64.9±16.9	p=NS
Gender, M/F	5/5	3/6	4/5	p=NS
Reason for ICU admission:				
Respiratory n=15	5 (50%)	4 (44.4%)	6 (66.7%)	
Cardiac n=3	1 (10%)	2 (22.2%)	0	
Neurological n=3	1 (10%)	1 (11.1%)	1 (11.1%)	
Trauma n=2	1 (10%)	0	1 (11.1%)	
Sepsis n=2	1 (10%)	0	1 (11.1%)	
Thoracics n=2	1 (10%)	1 (11.1%)	0	
Overdose n=1	0	1 (11.1%)	0	
APACHE II[2]	19.10±4.15	17.33±4.36	15.89±4.17	p=NS
BMI[3]	23.5±5.8	28.8±7.6	25.8±5.2	p=NS
Number of types of antibiotics/d,	1.5±0.9	1.3±0.8	1.4±1.0	p=NS
Survival				
ICU	9/10	8/9	8/9	p=NS
Hospital	8/10	5/9	8/9	
LOS[4]-ICU days	9.1 ±4.4 (4.0-19.0)	28.3±40.2 (2.5-127.5)	12.5±7.4 (4.0-27.0)	p=NS
LOS-hospital days	27.4±20.6[a] (5-70.5)	62.4±48.8[b] (10.5-151)	25.2±17.0[a] (6-66)	p=0.046

[1]Data expressed as mean ± standard deviation (range); [2]APACHE II= acute physiology and chronic health evaluation II; [3]BMI= body mass index calculated by: wt (kg)/ht(m^2);

[4]LOS=length of stay.

[ab]Columns with different letters are significantly different ($p<0.05$)

Table 2: Nutritional parameters of study participants by treatment group

	Live probiotics n=10	Sonicates n=9	Placebo n=9	Significance
SGA[1]: A	7	5	6	
B	2	3	3	
C	1	1	0	
Energy intake, kcals/d	1199±509[2]	1388±417	1406±261	p=NS
% energy requirements met[3]	74.6±13.28[a]	82.6±22.8[ab]	87.3±10.4[b]	P=0.034*
Protein Intake, g protein/d	56.0±23.6	64.8±19.1	65.7±12.3	p=NS
% protein requirements met[4]	64.5±18.81	74.33±19.59	87.3±10.4	p=NS

[1]SGA= subjective global assessment with A=well-nourished, B=moderately malnourished and C=severely malnourished. [2]Data expressed as mean ± standard deviation. [3]Percent energy requirements met determined by energy intake from enteral nutrition ÷ energy requirements assessed through indirect calorimetry. [4]Percent protein requirements met determined by grams protein consumed via enteral nutrition ÷ grams protein required from formulaic assessment of 1.2-1.5g protein/kg/day. [ab] Columns with different letters are significantly different.

Table 3: Effect of treatment and time on serum IgG levels.

	Live Probiotic N=9	**Sonicates** N=9	**Placebo** N=8	Effect of time p-value	Effect of treatment p-value
IgG Day 1 (g/l)	5.74±1.48[1]	7.90±2.50	6.99±2.08	p=0.026	p=0.367
IgG Day 7[2] (g/l)	7.38±2.19	8.03±2.69	7.47±2.06		

[1]Data expressed as means ± standard deviation. [2]Day 7 data, or at completion of study if completed prior to Day 7. Normal IgG levels 6.94-16.18g/l (adults).

Table 4: Effect of treatment and time on serum IgA levels.

	Live Probiotic n=9	**Sonicates** n=9	**Placebo** n=8	Effect of time p-value	Effect of treatment p-value
IgA Day 1 (g/l)	1.94±1.19[1]	2.43±2.49	1.91±0.86	p=0.021	p=0.812
IgA Day 7[2] (g/l)	2.61±1.61	2.57±1.84	2.11±0.79		

[1]Results expressed as means ± standard deviation. [2]IgA level at Day 7 or at completion of the study if study concluded prior to Day 7. Normal IgA levels 0.70-4.00g/l (adults).

Table 5: Effect of time and treatment on serum C-Reactive Protein levels.

	Live Probiotic n=9	**Sonicates** n=9	**Placebo** n=8	Effect of time p-value	Effect of treatment p-value
CRP Day 1 (mg/l)	112.5±94.3[1]	129.1±88.1	145.4±88.9	p=0.003	p=0.932
CRP Day 7 (mg/l)	103.0±68.7	66.1±64.8	72.6±74.3		

[1]Data expressed as means ± standard deviation. [2]CRP level at Day 7 or on completion of the study if completed prior to Day 7. Normal CRP levels 0.0-7.9 mg/l (adults).

Table 6: Incidence of diarrhea expressed as percentage of EN days with diarrrhea.

	Live probiotics n=9	**Sonicates** n=9	**Placebo** n=9	**Effect of treatment p-value**
% of EN days with diarrhea[1]	14.4±16.2[2]	11.1±15.6	22.7±25.6	p=0.447

[1]Results calculated by number of days with Hart & Dobb Score of 12 or greater divided by the number of days patient receiving treatment and enteral nutrition. [2]Data reported as percent ± standard deviation.

Table 7: Effect of time and treatment on MODS scores between Day 1 and Day 4, and Day 4 and Day 7.

	Live probiotics n=9	**Sonicates** n=8	**Placebo** n=9	Effect of Time p-value	Effect of Treatment p-value
MODS[1] Day 1	4.6 ± 4.1[2] (2.6 - 6.5)	4.0 ± 1.8 (2.0 – 6.0)	3.8 ± 1.6 (1.9 – 5.7)	p=0.908	p=0.930
MODS Day 4	3.4 ± 2.7 (2.1 – 4.8)	4.63 ± 1.30 (3.2 – 6.1)	4.1 ± 1.5 (2.8 – 5.5)		

	Live probiotics n=5	**Sonicates** n=7	**Placebo** n=6	Effect of Time p-value	Effect of Treatment p-value
MODS Day 7[3]	4.0 ± 1.9 (2.2 – 5.8)	3.7 ± 2.1 (2.2 – 5.3)	4.2 ± 1.6 (2.5–5.8)	P=0.558	P=0.243

[1]MODS = multiple organ dysfunction syndrome. Calculations for MODS score detailed in "methodology". [2]Data expressed as means ± standard deviation (range). [3]Day 7 compared with Day 4 only.

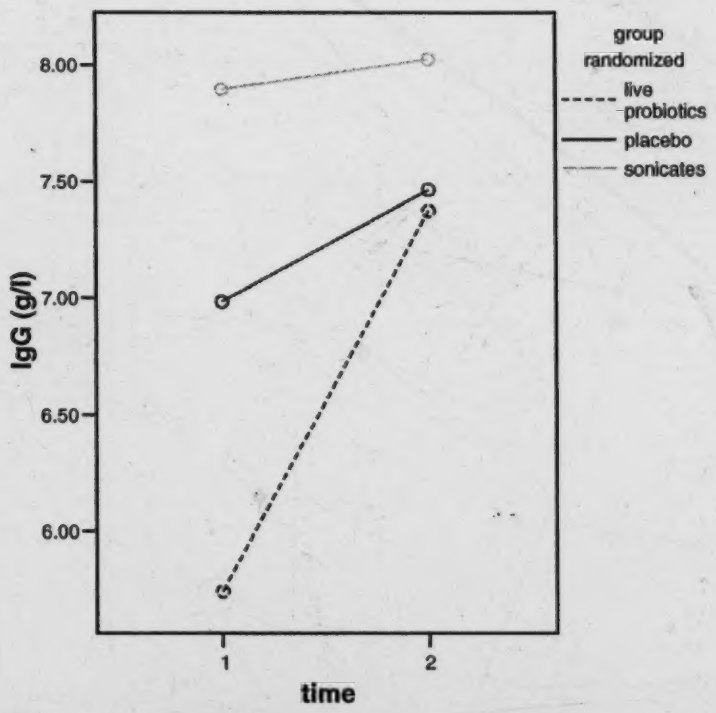

Figure 1: Effect of treatment and time on serum IgG. Data are expressed as means. Time 1 indicates IgG measurement on Day 1 prior to enteral nutrition and treatment. Time 2 indicates IgG measurement at Day 7 or at completion of study if subject discontinued prior to Day 7. No significant differences amongst treatments were identified by repeated measures ANOVA.

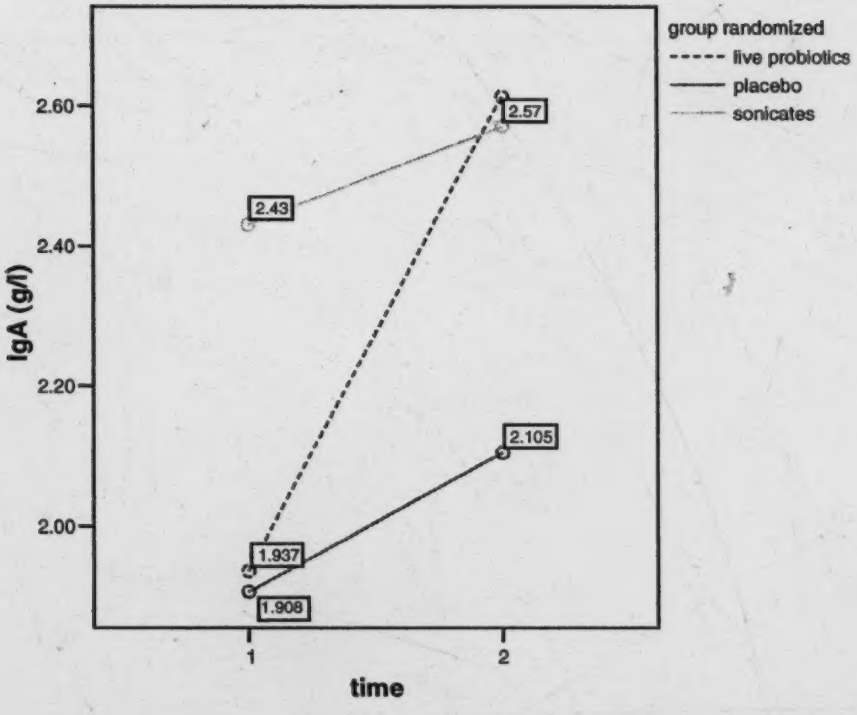

Figure 2: Effect of treatment and time on serum IgA. Data are expressed as means. Time 1 indicates IgA measurement on Day1 prior to enteral nutrition and treatment. Time 2 indicates IgA measurement at Day 7 or completion of the study. No significant differences amongst treatments were identified by repeated measures ANOVA.

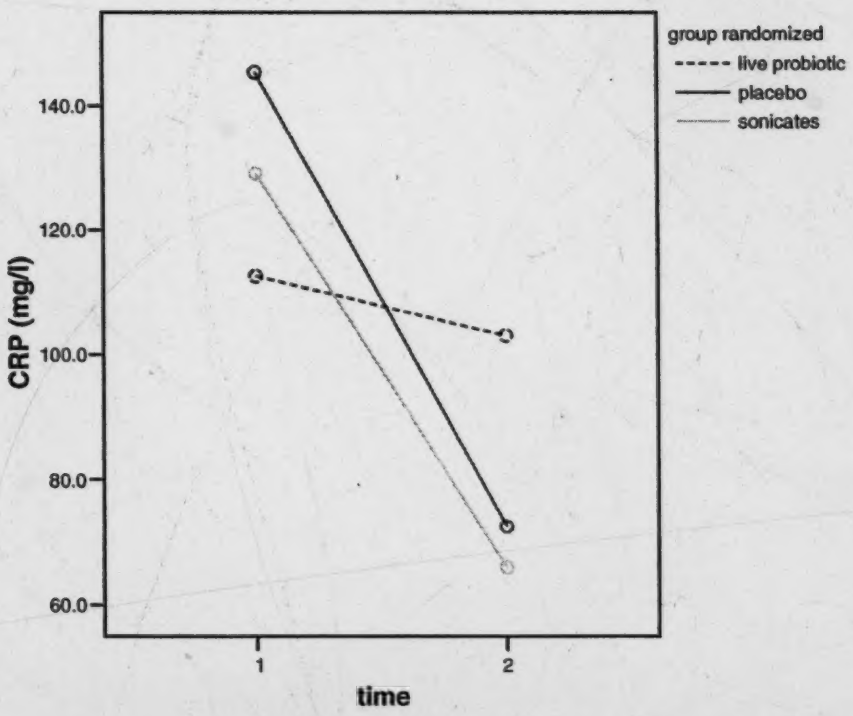

Figure 3: Effect of treatment and time on CRP levels. Data are expressed as means. Time 1 indicates CRP measurement on Day 1 prior to enteral nutrition and treatment. Time 2 indicates CRP measurement at Day 7 or at completion of the study if discontinued prior to Day 7. No significant differences amongst treatments were identified by repeated measures ANOVA. Abbreviation: CRP=C-reactive protein

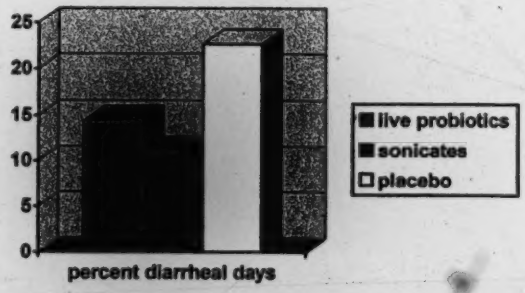

Figure 4: percent of diarrheal days calculated by number of days Hart & Dobb score >12 ÷ number of days enrolled in study. Data expressed as means of percentage. No significant differences noted between treatment groups.

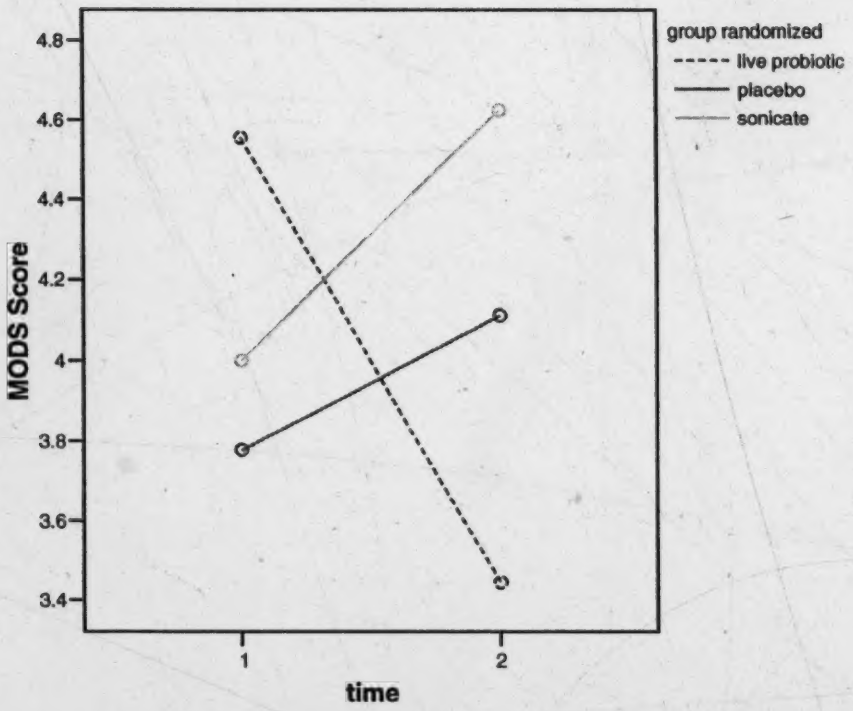

Figure 5: Effect of treatment and time on development of MODS. Data are expressed as means. Time 1 indicates MODS score on Day 1 prior to enteral nutrition and treatment. Time 2 refers to MODS scores on Day 4. No significant differences amongst treatments noted by repeated measures ANOVA.

Abbreviation: MODS=multiple organ dysfunction syndrome.

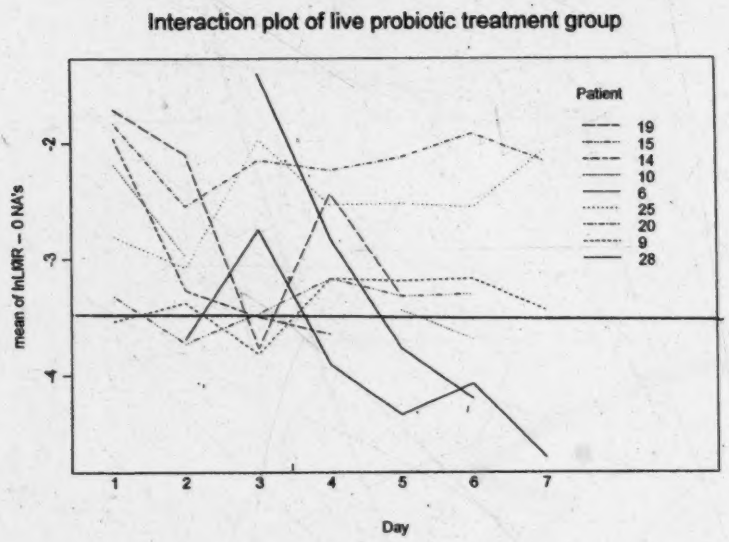

Figure 6: Intestinal permeability of individual subjects receiving live probiotic treatment on Days 1-7; n=9. Abbreviations: lnLMR= log of lactulose/ mannitol ratio. Solid horizontal line shows upper limit of normal (-3.50).

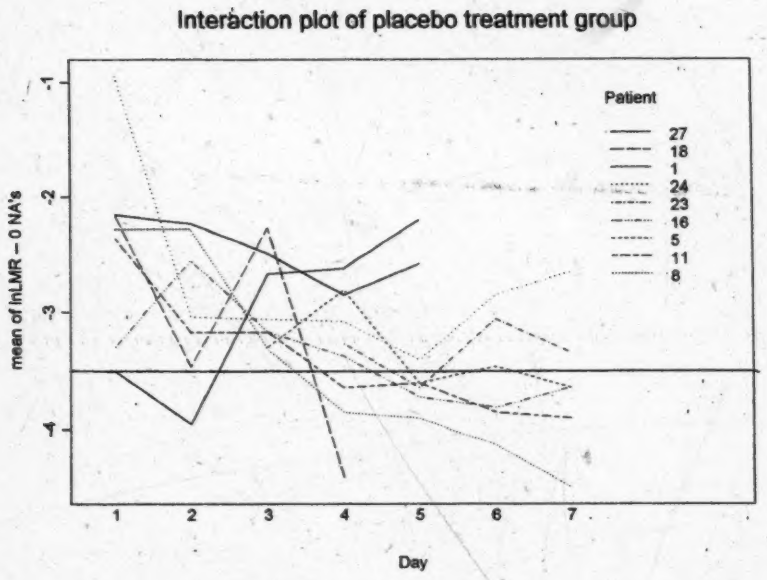

Figure 7: Intestinal permeability of individual subjects receiving placebo treatment on Days 1-7; n=9. Abbreviations: lnLMR= log of lactulose/ mannitol ratio. Solid horizontal line shows upper limit of normal (-3.50).

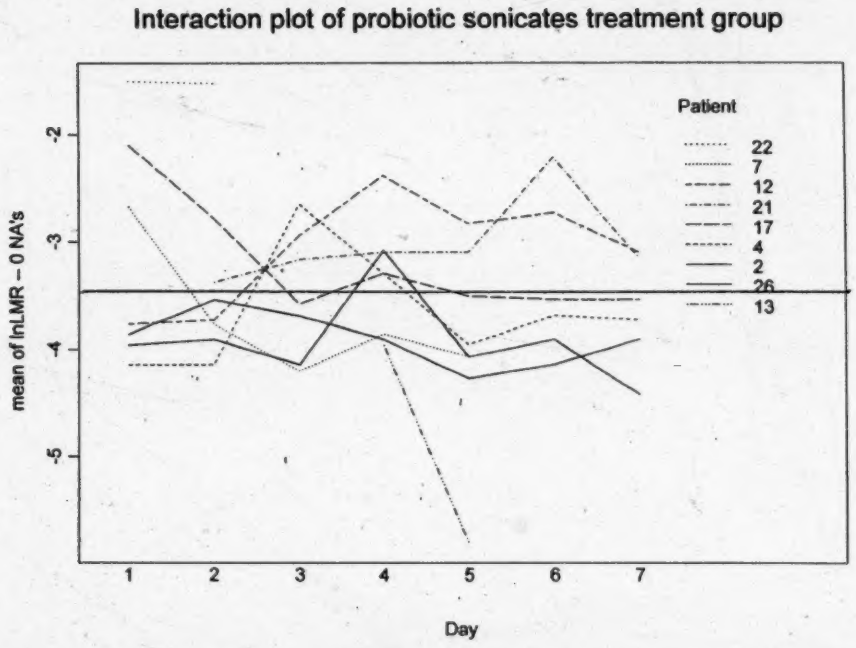

Figure 8: Intestinal permeability of individual subjects receiving probiotic sonicates on Days 1-7; n=9. Abbreviations: lnLMR= log of lactulose/ mannitol ratio. Solid horizontal line shows upper limit of normal (-3.50).

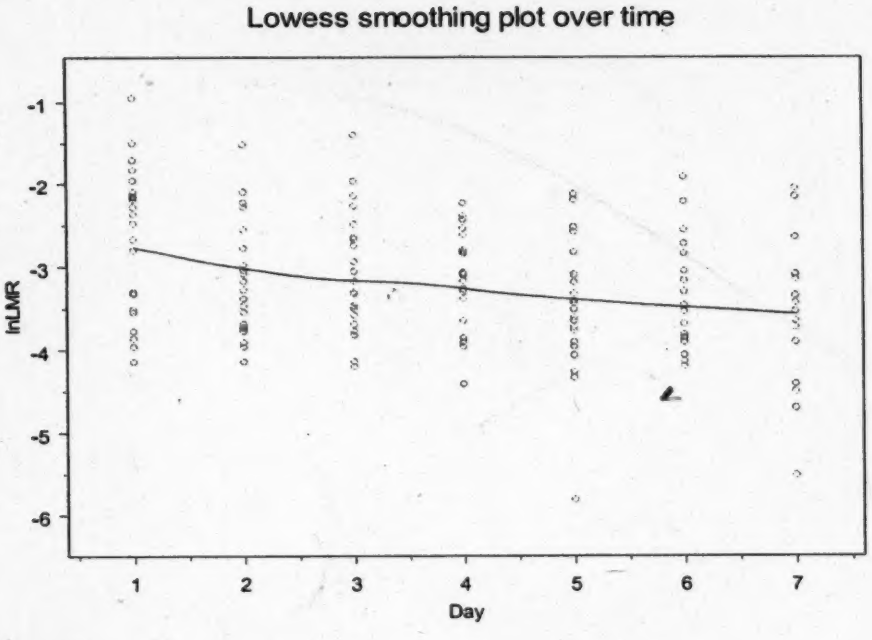

Figure 9

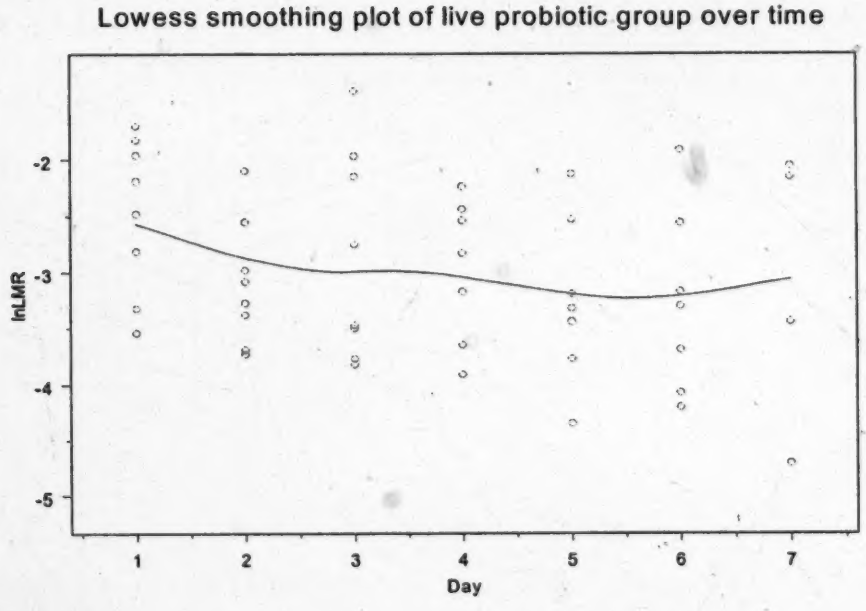

Figure 9a

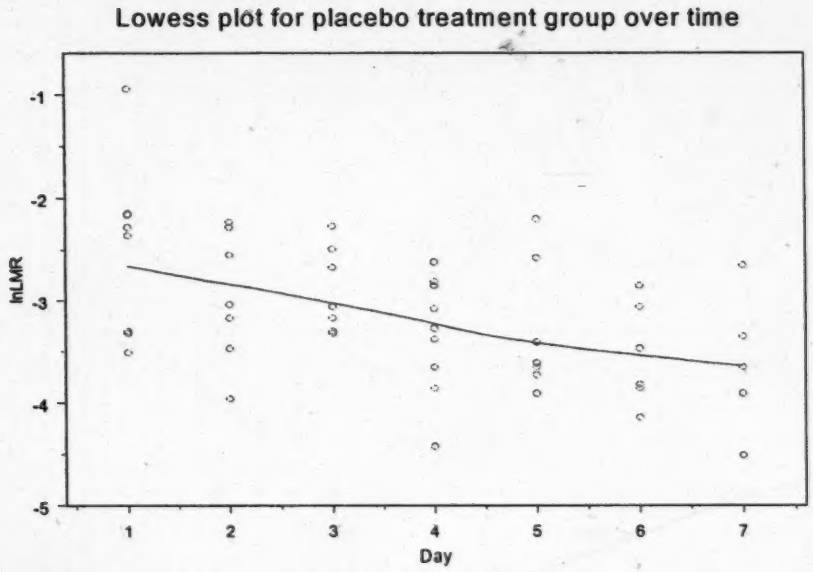

Figure 9b

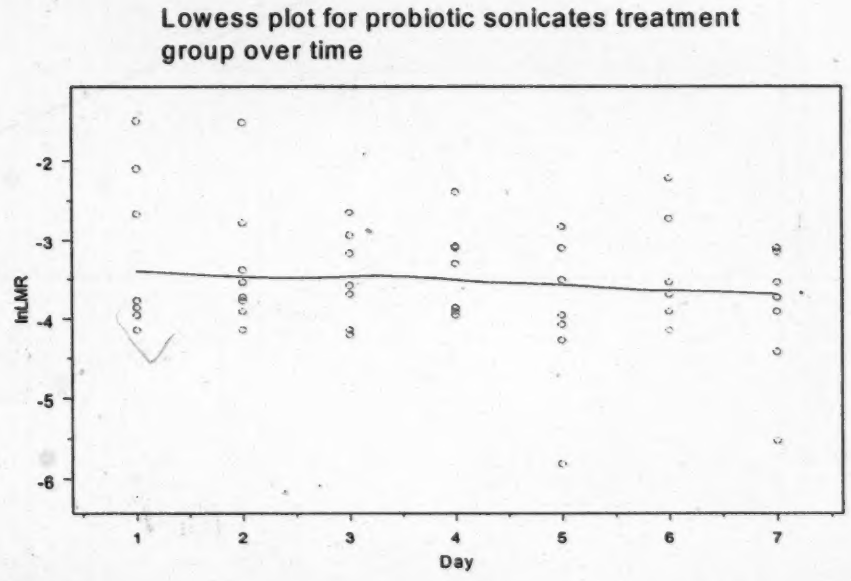

Figure 9c

Smoothing plots of treatment groups over time (Figures 9a-9c) (not significant) and all groups (Figure 9) (p=0.023). Abbreviations: lnLRM=log of lactulose/ mannitol ratio. Normal lnLMR –3.5 or less

Chapter Five

Discussion

A. Introduction

Although statistical signficance was not achieved on the outcomes of the current study, it is worthy to note that the patients who received live probiotics showed trends toward a greater increase in IgA and IgG concentrations, decreased incidence of diarrhea, decreased MODS score between Day 1 and 4, and a decreased length of ICU stay. These changes occurred despite this treatment group having the highest severity of illness scores and the greatest degree of organ dysfunction, as well as being prescribed a slightly larger number of antibiotics. Also of note, is that the patients randomized to receive probiotic sonicates had the lowest incidence of diarrhea and the lowest intestinal permeability, suggesting that probiotics can have clinical impact in a non-viable form. It is also noteworthy that no side effects of the probiotics were observed by either research or nursing staff.

Compelling evidence exists to demonstrate the efficacy of probiotics in the treatment of acute diarrhea and as an adjunct therapy in inflammatory bowel disease (Isolauri et al, 1991; Venturi et al, 1999). The potential of probiotics to reduce the incidence of allergic disease and to enhance the immune response to infections is being investigated and provides support for the arguments for widespread use of probiotics in healthy populations (Vanderhoof, 2001). The vast majority of studies demonstrating beneficial effects of probiotics in the clinical setting have used *Lactobacillus* GG, *Lactobacillus plantarum* 299, or VSL#3.

The application of probiotics to the critical care environment has received limited attention to date. The potential clinical effects of probiotics in the intensive care unit include prevention or reduction of duration of diarrhea, enhancement of the immune system, and reduction of the incidence and severity of sepsis (Bengmark, 2002). The reduction of commensal gut flora occurs early in critical illness. Contributing factors include lack of normal food and inhibition of gastrointestinal secretions, along with strict adherence to hygienic principles. Tube feeding-related diarrhea, largely associated with antibiotic usage, and low serum albumin levels, is common-place in the ICU (Guenter et al, 1992). Guenter estimated diarrhea rates of 30% in ICU patients receiving tube feeds. A strong trend toward use of enteral nutrition rather than parenteral nutrition has already been observed, along with decreased usage of H_2-blockers, and proton pump inhibitors (Bengmark, 2002). Furthermore, concern has been expressed as the degree of microbial resistance to indiscriminately prescribed antibiotics increases.

The purpose of this study was to determine the effects of live probiotics, VSL#3, and a bacterial sonciate of VSL#3 on the reduction of multiple organ dysfunction syndrome in critically ill enterally fed patients.

B. Study Participants

There was no significant difference in length of ICU stay for the three treatment groups. The length of hospital stay and ICU stay was longest for the patients randomized to the sonicate group (62.4 days for hospital stay and 28.3 days for ICU stay). The data for this sub-group is skewed by the 2 patients with hospital lengths of stay greater than 100 days. Due to the short-term impact of

probiotics, it is unreasonable to make any correlation between the lengthy hospital stays with probiotic sonicate treatment during the first week of ICU admission. Average length of stay in the Royal Alexandra Hospital ICU is eight days. Although the patients randomised to receive live probiotics had the highest severity of illness with an APACHE II score of 19.10, it is interesting to note that they also had the shortest length of ICU stay (9.1 days). APACHE II scores of 15 to 19 correlate with a 23% mortality rate in the ICU (Knaus et al, 1985).

C. Nutritional Parameters

The percentage of energy requirements achieved with enteral nutrition was significantly greater for the placebo group (87.3%) than for the sonicate group (82.6%), and the live probiotics group (74.6%) ($p=0.034$). Difficulties establishing enteral nutrition are greater in patients with higher severities of illness due to gut dysmotility and hypoperfusion to the gastrointestinal tract (Moore & Weisbrodt, 2003). The most frequent reasons for stopping or interrupting enteral feeds were for medical procedures and for high gastric residuals. Two of the 28 patients received less than 50% of the prescribed energy and protein provisions.

D. Immunological Parameters

The immune system consists of specific and non-specific components that have distinct, yet overlapping functions. These two entities are known as the adaptive and the innate immune systems. The adaptive immune system consists of the humoral and cell-mediated immune systems, which provide specificity and a memory of previously encountered antigens. The innate immune system lacks

the specificity of the adaptive immune system, however the components of the innate system are essential because they are responsible for the natural immunity to a vast array of environmental microorganisms (Kircher & Marquardt, 2002). Adaptive immunity involves B and T lymphocytes for their recognition of antigen. Once B cells recognize antigens, they are programmed to produce and release antibodies. Antibodies, or immunoglobulins, are capable of surrounding the antigen and essentially, flagging it for easier recognition by phagocytic cells. Immunoglobulins can also surround antigens in such a way as to render them inactive by preventing adherence to additional host cells (Lentz & Feezor, 2003). Humoral, or antibody-mediated immunity is the primary defense against bacterial invasion (Kircher & Marquardt, 2002). Care in the ICU setting is associated with dramatic changes in nutrition, along with changes in physicial activity, body temperature, sleep, mood, and circadian rhythm. All of these factors combined with the increased consumption of drugs has a profound negative influence on the immune response of the patient (Bengmark, 2002).

1. Immunoglobulin G (IgG)

Immunoglobulin G (IgG) is released from B cells and functions as the predominant immunoglobulin found in serum. The ability of IgG to diffuse into body tissue facilitates the efficient elimination of antigens. Elevated IgG levels are found in patients with diffuse liver disease, autoimmune diseases, sarcoidosis, lymphoid malignancies and multiple myeloma. Although elevated levels are undesirable, so too are depressed levels as they can be indicative of protein-losing enteropathies and malabsorption syndromes that can leave a patient vulnerable to

infections. All Day 1 IgG levels were within or below the normal reference range of 6.94-16.18g/l for adults. The results show a statistically significant increase in IgG levels over time in all enrolled patients ($p=0.026$). Although not statistically significant, the increase in IgG levels in the live probiotics group was observed to be nearly 3½ times the increase seen in the placebo group. Observed power for IgG levels amongst treatment groups over time was 0.395, indicating the need for a larger sample size to make definitive conclusions. Since the live probiotics group had the lowest initial IgG levels, and previous research shows that probiotics are most effective in patients who are immune-compromised (Gill et al, 2001), perhaps this is a reflection of immune-enhancement in this specific patient group.

2. Immunoglobulin A (IgA)

SIgA is the primary immunoglobulin of all mucosal surfaces and exocrine secretions. This immunoglobulin plays a large role in defense against organisms that invade the mucosal surfaces. SIgA has been found to possess antibody activity against viruses and several bacteria, especially the gram negative bacteria in the gut (Lentz & Feezor, 2003; Kircher & Marquardt, 2002). Research has demonstrated the ability of bifidobacteria to increase production of IgA in tissue extracts of the small intestine (Yasui et al, 1992). Of the 120 strains of bifidobacteria tested, only 3 strains were shown to induce production of IgA. Two strains that have this effect are *Bifodbacterium* breve and *Bifidobacterium* longum, both of which are components of VSL#3. Some lactic acid bacteria have also been shown to enhance production of IgA. *Lactobacillus* GG has been

shown to significantly increase the IgA response in Crohn's disease (Malin et al, 1996), and to enhance the IgA response to rotavirus (Kaila et al, 1992). In this study, IgA levels increased significantly in all groups from Day 1 to Day 7 ($p=0.021$). Although not statistically significant, the increase of IgA levels in the live probiotic group was more than 3-fold the increase in IgA levels in the placebo group. The increase in IgA levels in the sonicate group was less than the placebo group, although initial IgA levels were higher in the bacterial sonicates treatment group. The increase in IgA levels corresponds to the increases noted in the Crohn's literature (Malin et al, 1996) and the pediatric literature (Kaila et al, 1992), although due to an observed power of 0.301, results were not statistically conclusive.

E. Inflammatory Response (C-Reactive Protein)

C-reactive protein (CRP) is an acute phase protein found in low concentrations in the serum of healthy individuals. Measurement of CRP aids in the evaluation of stress, trauma, infection, inflammation and surgery. The appearance of CRP in the serum is a non-specific phenomenon which can be found in all acute inflammatory diseases, and also with malignant tumors. In this study, CRP levels decreased significantly over time ($p=0.003$) for all patients, however there was no significant difference between treatment groups. Figure 3 demonstrates that the decrease over time of the placebo and sonicates group was more dramatic than the decrease in CRP levels of the live probiotics group. The range in CRP levels on Day 1 was extreme (22.3-346.0). CRP's rapid increase in synthesis within hours after tissue injury or infection suggests that it contributes to

host defense, and that it is part of the innate immune response (Black et al, 2004). A recent review suggested that CRP is protective against a variety of bacterial invasions in the animal model, and that it is likely that the activity of CRP in humans can be pro-or anti-inflammatory (Black et al, 2004). Literature has shown that CRP concentrations increase with the number of cardiovascular risk factors, so the increase in CRP levels in many of the live probiotics subjects may also be an indication of ongoing cardiac events (McDonald et al, 2004). However, the literature does report that some probiotic strains can contribute to chronic inflammation while other strains may suppress the inflammatory response (Madsen, 2001; Isolauri et al, 2001).

F. Incidence of Diarrhea

The cause of diarrhea in the intensive care unit is multifactorial. Guenter et al (1992) showed that 41% of ICU patients who received antibiotics develop diarrhea. Although patients with diarrhea had significantly greater hypoalbuminemia, antibiotic usage was the factor most strongly associated with diarrhea during tube feedings (Guenter et al, 1992). Probiotic treatment has been proven effective in both the prevention and treatment of various forms of diarrhea. In the current study, efforts were made to make a stringent measurement of diarrhea by implementation of the Hart and Dobb Scale. The placebo group demonstrated a 22.7% incidence of diarrhea compared to the sonicates group who experienced an 11.1% incidence of diarrhea, and the live probiotics group who experienced diarrhea on 14.4% of enteral feeding days. Although not statistically significant, the decreased incidence of diarrhea in both the live probiotics and

sonicates treatment groups is noteworthy. Absolute power of 0.176 was low as well as the effect size of 0.065. In a study by Bleichner et al, a total of 128 patients were randomized to receive one of placebo or *Saccharomyces boulardii* with their tube feedings. Treatment with *S. boulardii* reduced the mean percentage of days with diarrhea per feeding days from 18.9% to 14.2%, p=0.0069 (Bleichner et al, 1997). With 64 patients per treatment group, the investigators concluded that S. boulardii prevents diarrhea in critically ill tube-fed patients, especially in patients with risk factors for diarrhea. Five independent factors were associated with diarrhea in a multivariate analysis: fever or hypothermia, malnutrition, hypoalbuminemia, previous suspension of oral feeding, and presence of an infection site.

G. Multiple Organ Dysfunction Syndrome (MODS)

The multiple organ dysfunction score was developed as an outcome measure that would correlate strongly with the ultimate risk of ICU mortality and hospital mortality. Changes in multiple organ dysfunction score reflect organ dysfunction developing during the ICU stay (Marshall et al, 1995). The research by Marshall et al has demonstrated that admission MODS scores along with increasing multiple organ dysfunction scores correlated with ICU and hospital mortality. Hospital mortality rate for patients with a MODS score of 1 to 4 was approximately 5%; hospital mortality for patients with a MODS score of 5 to 8 was approximately 15%. Patients who were admitted with a MODS score of 21 to 24 in the Marshall study had close to a 100% mortality rate. The patient population in this study had calculated mean MODS scores of 3.78 (placebo

group) to 4.56 (live probiotic group). This study showed a decrease in MODS scores in the patients randomized to receive live probiotics, and an increase in MODS scores in the patients randomized to receive placebo or sonicates. Although not statistically significant, it is interesting to note that the MODS score at admission to the study correlates well with the APACHE II severity of illness scores. The highest APACHE II scores on Day 1 were found in the patients randomized to receive live probiotics while the lowest APACHE II scores were found in the patients receiving the placebo. Although the patients which received live probiotics had a slightly higher severity of illness as demonstrated by APACHE II scores, and scored a higher organ dysfunction, 4 of the 9 patients were well enough to be extubated and have enteral feeding discontinued before Day 7 (44%). In the placebo group, 3 of the 9 patients (33%) were deemed ready to be discontinued from enteral feeding. Of the patients who remained in ICU past 7 days, MODS score increased for the placebo and the live probiotics group, but decreased for patients in the sonicates group. Changes to the MODS scores were not statistically significant for time or treatment.

The range of MODS scores for the cohort of patients in this study was 1.56 to 6.47. Considering that MODS scores can be as high as 24, the exclusion criteria for this study eliminated patients with the higher MODS scores. A larger range of MODS scores on admission to the study may have shown greater changes with treatment and time.

H. Intestinal Permeability

Intestinal permeability was not affected by live probiotic treatment, however there was a significant decrease in intestinal permeability in all treatment groups over the seven days of treatment. Since the majority of patients demonstrated reduced inflammatory response, enhanced immune function, and decreased MODS score, and in fact, survived their ICU admission (25/28), it intrinsically makes sense that intestinal permeability would also improve. Patients randomized to probiotic sonicates demonstrated reduced intestinal permeability, however, initial intestinal permeability results were within the normal range (less than –3.5). In contrast, the patients randomized to the live probiotic group and placebo group had an abnormally increased intestinal permeability at baseline. Doig et al (1998) showed that critically ill patients with an intestinal permeability greater than –2.51 were more likely to develop MODS. The subject who was switched to total parenteral nutrition due to a bowel obstruction was randomized to the live probiotic group. This subject was not dosed with live probiotics according to protocol, nor was the subject able to tolerate enteral nutrition at any point during the study. Linear mixed effects allows comparisons between the means of cohorts, though they may differ. However, it is difficult to determine if the sonicates were effective in decreasing, or maintaining, permeability, or if the results are a reflection of the baseline values. Interestingly, the patients that received bacterial sonicates also had the lowest MODS scores by Day 7.

The patients who tolerated higher energy intakes from enteral nutrition also demonstrated a significantly increased intestinal permeability. There are two possible explanations for this observation. Firstly, increased energy intake

correlates with increased volume of intestinal fluids which may impact intestinal permeability. For accurate interpretation, research would need to be completed to compare intestinal permeability of critically ill patients receiving TPN to patients receiving enteral nutrition. A study published by Kompan et al showed that patients started on enteral nutrition 24 hours or more after admission to ICU demonstrated increased intestinal permeability by Day 2. In order to decrease intestinal permeability, enteral nutrition needed to be started within 6 hours of ICU admission (Kompan et al, 1999). No patients in this study were initiated on enteral nutrition within 6 hours of ICU admission. The second explanation could be related to the impaired mucosal perfusion described in ICU patients. During periods of hypoperfusion common in the first 48 hours of ICU admission, the gut mucosa appears vulnerable to injury (Moore & Weisbrodt, 2003). It has been suggested that fibre can compromise the mileau of the gut in hemodynamically unstable patients (McClave & Chang, 2003). In this study, as energy intake increased, fibre intake also increased.

I. General Discussion

The present study employed a double-blind, placebo-controlled randomized design in order to determine the effects of live and sonicated probiotics on immune and inflammatory function, incidence of diarrhea, intestinal permeability and development of multiple organ dysfunction in critically ill patients. Implementation of the study was made possible through the coordinated effort of 8 different hospital departments. Of the 28 patients recruited, one subject did not receive appropriate dosing of the treatment, and this was related to the

development of a bowel obstruction over the course of the study, preventing nasogastric drug administration. One member of the research team was available to nursing staff for consultations on a 24-hour basis, seven days a week. Due to the close monitoring of all study patients and computerized verification of the study treatment, compliance to the protocol was excellent. Ideally, all patients would have remained in the study for 7 days, however due to the uncontrollable variables of hospital treatment, this would be virtually impossible to predict upon admission to ICU.

The absolute power and effect size were low in all outcome measures indicating that the study needs to be replicated in a larger sample size to be conclusive. The second limitation of the study was the heterogeneity of the ICU patients enrolled. Study subjects included medical, surgical, and trauma patients, and in a study of this size, the variability in baseline data made statistical interpretation with conclusive results challenging.

There is considerable variation in the literature with respect to probiotics species and dosing. The success of one probiotic species in a certain application does not imply that all related strains or doses of this species will be capable of producing a comparable response in a different environment (Vanderhoof, 2001). Recent literature suggests that probiotic bacteria may exert some beneficial effects, even when given in a nonviable form. (Rachmilewitzetal, 2004).

Although statistical signficance was not achieved on the outcomes of the current study, it is worthy to note that the patients who received live probiotics had a greater increase in IgA and IgG concentrations, decreased incidence of

diarrhea, and a decreased length of ICU stay. These changes occurred despite this treatment group having the highest severity of illness scores and the greatest degree of organ dysfunction, as well as being prescribed a slightly larger number of antibiotics. Also of note, is that the patients randomized to receive probiotic sonicates had the lowest incidence of diarrhea, suggesting that probiotics can have clinical impact in a non-viable form.

Inflammatory function was a confounding factor in the current study as CRP levels increased in over half of the patients on live probiotics. This may be explained by the number of cardiac events that occurred in the patients who received live probiotics. In a recent review by Black et al (2004), the authors discuss that CRP may actually contribute to host defense, defending against bacterial invasions. Thereby, an increased CRP level is found to be beneficial outcome.

Intestinal permeability was not altered by the addition of live probiotics to enteral feeds. Any alterations in immune function, incidence of diarrhea, or change in MODS score seen in the live probiotic treatment group cannot be attributed to intestinal permeability changes. Although not statistically significant, patients that received probiotic sonicates demonstrated decreased intestinal permeability, along with a decreased incidence of diarrhea and lower MODS score by study conclusion. These results support the animal data, suggesting that protective effects of probiotics may be mediated by their own DNA. Sonicates contain cell fragments, proteins and DNA. However, it is unknown whether probiotic sonicates would exert the same effects in patients who have an increased

intestinal permeability at baseline. Future studies may consider attempting to enrol patients with a higher severity of illness; as well, colonic permeability rather than intestinal permeability may be of greater significance, as prebiotics and probiotics have the greatest synbiotic impact at the level of the colon.

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Appendix A
Study Information and Consent Form

Capital Health

Healthier people in healthier communities

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Family Information Sheet
Probiotic therapy in critically ill enterally fed patients

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Purpose: The purpose of this study is to see if patients who are taking a diet containing probiotics develop fewer complications while in the intensive care unit. Probiotics are bacteria, which have been shown to have positive health benefits in people who are healthy and in people who are ill. The most common probiotic that you are likely familiar with is the probiotic that you eat when you eat yogurt. It is healthy to have bacteria in our system to fight off infections and prevent inflammation. When we get sick, drugs or disease may destroy the "good" bacteria. Probiotics provide a source of the "good" bacteria to the gut.

Background: Nutrition has been shown to play an important role in the recovery of a patient who is critically ill. Patients who are admitted to the intensive care unit are started on liquid nutrition about 1 to 2 days after admission. Nutrition is given via a tube through the nose and into the stomach. In most cases, your relative already has the tube in place. Studies are showing that probiotics may help to decrease infection and inflammation in ill patients. Diarrhea is a common side effect of tube feedings in the intensive care unit. Probiotics may help to decrease the amount of diarrhea that occurs.

Procedures: If you agree to have your relative participate, he/she will randomly receive liquid nutrition with a probiotic or liquid nutrition without the probiotic. Some patients will receive a probiotic with live organisms; others will receive a probiotic compound where the organisms are no longer alive. Each of the groups will have two blood tests to show us the degree of inflammation and immune function. It is unlikely that extra blood (beyond standard procedures) will need to be taken in order for us to do the tests. They will also undergo a breathing test, which shows how many calories we need to feed them. All of these tests are common for a patient in the intensive care unit. In addition, a 6-hour urine collection will occur daily for seven days. The urine tests show the permeability of the intestine to sugar. Intestinal permeability is a marker for infection,

and for risk of complications. The patient's bloodwork (Hemoglobin, White Blood cells, and electrolytes) results will be reviewed by the research dietitian. The maximum amount of time your relative would be in the study is seven days. If they are able to start eating prior to seven days, or are ready to be transferred out of intensive care, the study will be discontinued.

Benefits: If your relative receives nutrition containing the probiotic, he/she may have reduced infection and inflammation. As well, diarrhea that can result from feeds and drugs, may be reduced.

Risks: There are no known risks to your family member receiving probiotics.

Confidentiality: Information from this study will be kept private. Your relative's name will not be used in any papers written about the study. Only group results will be reported. The investigators listed above will have access to your relative's medical records.

Voluntary Participation and Withdrawal: Taking part in this study is voluntary. If you choose to withdraw from the study, you may do so with no adverse effects. Your relative's care will not be affected.

Additional Contacts: If you have any concerns about any aspect of this study, you may contact any of the investigators.

If you have concerns about your rights in this study, please call the Capital Health Authority Patient Relations Office, at (780) 407-1040. This office has no connection to the study or the research staff.

Capital Health

Healthier people in healthier communities

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CONSENT FORM

Part 1

Title of Project: Probiotic therapy in critically ill enterally fed patients

Principal Investigator(s): Dr. Leah Gramlich, Gastroenterology & Nutrition 421-1029

Co-Investigator(s): Cathy Alberda, Clinical Nutrition 477-4439 Dr. Jim Kutsogiannis, Critical Care 491-5387
Dr. Linda McCargar Dr. Karen Madsen Dr. Catherine Field Dr. Jon Meddings

Part 2

Do you understand that your family member has been asked to be in a research study?	Yes	No
Have you read and received a copy of the attached Information Sheet?	Yes	No
Do you understand the benefits and risks involved in taking part in this research study?	Yes	No
Have you had an opportunity to ask questions and discuss this study?	Yes	No
Do you understand that you are free to refuse to participate or withdraw from the study at any time? You do not have to give a reason and it will not affect the care of your family member.	Yes	No
Has the issue of confidentiality been explained to you? Do you understand who will have access to your records?	Yes	No

This study was explained to me by: _____ _____

I agree that my family member will take part in this study.

_____ _____ _____
Signature of Family Member Date/Time Witness

_____ _____
Printed Name Printed Name

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

_____ _____
Signature of Investigator or Designee Date/Time

THE INFORMATION SHEET MUST BE ATTACHED TO THIS CONSENT FORM AND A COPY GIVEN TO THE RESEARCH SUBJECT

Appendix B
Preparation of Bacterial Sonicates

Procedure for Preparation of Bacterial Sonicates

1. **Grow bacteria overnight**

2. **Centrifuge liquid culture @ 3000 rpm for 10 minutes**

3. **Discard the supernatant and wash the pellet in 15 ml Phosphate Buffered Saline**

4. **Pellet cells @ 3000 rpm for 10 min and re-suspend pellet in appropriate volume of sonication buffer (mono Q)**

5. **Take a 50 µl sample before sonication and add 5 µl of 1N NaOH (to yield a final concentration of 0.1N NaOH). Heat the sample to 80°C for 1 hour to allow the proteins to be released from the sample. Before analying the protein, centrifuge sample @ 5000 rpm for 10 min. to clarify the sample**

6. **Sonicate the samples. Use setting µ5 (micro tip limit) and sonicate each sample 30 sec x 3 on ice with small rests in between so that the samples stay cold**

7. **Centrifuge samples in the Micro centrifuge @ 5000 rpm for 10 minutes. Collect the supernatant**

8. **Filter using millex-HV filter units 0.45µM**

9. **Analyze final protein content with Bio-Rad protein assay**

Appendix C
Procedure for Performing Lactulose Mannitol Ratios

Procedure for Lactulose Mannitol Ratios for ICU Patients

1. Measure 7.5 ml of Lactulose into a 10 ml syringe and 2 grams of Mannitol into container. Send to nursing unit. *

2. Make up 10% Thymol in70% Iso-propyl alcohol. Store at 2-8° C. (Stable for 1 week). *

3. Add 5 ml of 10% Thymol into the urine bottle. *

4. Add 80 ml of Gentamicin to urine bottle. *

5. Reconstitute Mannitol with 20 ml sterile water. Bolus both the Mannitol and Lactulose into NG/OG/NJ/keofeed tube; flush with 20 ml sterile water after administration of both meds. ***

6. Collect urine for 6 hours. (Maintain on ice). ***

7. Measure urine volume, mix, and take two 15 ml samples. Freeze within 24 hours. **

8. Store urine at -70° C. **

9. Send 1 aliquot of urine on dry ice (via courier) to U of Calgary. Maintain 1 sample as back-up at University of Alberta -70°C. (Study investigator)

10. Analyze within 2 months. (Dr. Medding's lab, U of C)

*Pharmacy

**Lab

***ICU Nurse

Appendix D
Enteral Nutrition Daily Energy & Protein Intakes

Probiotics Nutrition Worksheet

Initials (FML) _ _ _ ICU ID Code _ _ _ Patient Number _ _
Ht (cm) _ _ _ Wt (kg) _ _ _ (actual/est) BMI* _ _
SGA on Day 1: A B C
Hours to initiation of EN: _ _

Energy Expenditure (based on metabolic cart) _____kcals Day __
Protein Requirements (calculated)_____g/day

Energy and Protein Intakes

Day (_ _-_ _ _-_ _) day-mon-year	20. Energy Intake (kcals/day)	22. Protein intake (g/day)
Mean:		
% of req'ts		

*BMI (wt in kg/ht in m^2)
% of energy requirements (average 7 day energy intake / measured energy expenditure)
% of protein requirements (average 7 day protein intake / calculated protein requirements)

Appendix E
Subjective Global Assessment

Subjective Global Assessment of nutritional status

History

1. Weight Change
   Overall loss in past 4 months:___kg%loss___
   Change in past 2 weeks:___increase___no change___decrease
2. Dietary intake change relative to normal
   No Change___
   Change: duration___weeks
   Type: sub-optimal solid diet___full liquid diet___hypocaloric Liquids___starvation___
3. Gastrointestinal symptoms persisting for 2 weeks
   None___Nausea___Vomiting___Diarrhea___Anorexia___
4. Functional Capacity
   No Dysfunction___
   Dysfunction: duration___weeks
   Type___working sub-optimally___ambulatory___bedridden___
5. Disease and its relationship to nutritional requirements
   Primary diagnosis: _____
   Metabolic demand/ Stress no___low___moderate___high___

Physical (for each specify: 0=normal, 1+=mild, 2+=moderate, 3=+severe)
Loss of subcutaneous fat (triceps, chest)___
Muscle wasting (quadriceps, deltoids)___
Ankle edema___Sacral edema___Ascites___

Subjective Global Assessment Rating
Well nourished A___
Suspected or moderately malnourished B___
Severely malnourished C___

Appendix F
Hart & Dobb Diarrhea Scale

Probiotic Therapy in Critically Ill Patients: Hart & Dobb Diarrhea Scale

ICU ID Code ___ Patient Number ___ Patient Initials ___ Date __-___-__
Shift: A or B

In order to determine objectively whether your patient has diarrhea, please circle a number each time your patient has a bowel movement.

Estimated volume, ml

Consistency	<200	200-250	>250
Formed	1	2	3
Semisolid	3	6	9
Liquid	5	10	15

Estimated volume, ml

Consistency	<200	200-250	>250
Formed	1	2	3
Semisolid	3	6	9
Liquid	5	10	15

Estimated volume, ml

Consistency	<200	200-250	>250
Formed	1	2	3
Semisolid	3	6	9
Liquid	5	10	15

Estimated volume, ml

Consistency	<200	200-250	>250
Formed	1	2	3
Semisolid	3	6	9
Liquid	5	10	15

Estimated volume, ml

Consistency	<200	200-250	>250
Formed	1	2	3
Semisolid	3	6	9
Liquid	5	10	15

Adapted from Hart & Dobb, 1988

Appendix G
Multiple Organ Dysfunction Score

Multiple Organ Dysfunction Score

Score 0 thru 4 for each organ system, based upon the following parameters. Add up the 6 scores to give the total MODS score.

Organ System	Score 0	Score 1	Score 2	Score 3	Score 4
Respiratory[a] (PO_2/FIO_2 ratio)	>300	226-300	151-225	76-151	<75
Renal[b] (serum creatinine)	<100	101-200	201-350	351-500	>500
Hepatic[c] (serum bilirubin)	<20	21-60	61-120	121-240	>240
Cardiovascular[d] (PAR)	<10.0	10.1-15.0	15.1-20.0	20.1-30.0	>30.0
Hematologic[e] (platelet count)	>120	81-120	51-80	21-50	<20
Neurologic[f] (Glasgow Coma Score)	15	13-14	10-12	7-9	<6

[a]The PO_2/FIO_2 ratio is calculated without reference to the use or mode of mechanical ventilation, and without reference to the use or level of positive end-expiratory pressure; [b]the serum creatinine concentration is measured in µmol/l, without reference to the use of dialysis; [c]the serum bilirubin concentration is measured in µmol/l; [d]the pressure-adjusted heart rate (PAR) is calculated as the product of the heart rate (HR) multiplied by the ratio of the right atrial (central venous)pressure (RAP) to the mean arterial pressure (MAP): PAR=HR x RAP/mean BP; [e]the platelet count is measured in platelets/ml 10^{-3}; [f]the Glasgow Coma Score is preferably calculated by the patient's nurse, and is scored conservatively (for the patient receiving sedation or muscle relaxants, normal function is assumed, unless there is evidence of intrinsically altered mentation).

Appendix H
Acute Physiology and Chronic Health Evaluation

Acute Physiology and Chronic Health Evaluation

A. Acute Physiology Score (12 variables)

Physiological Variable	+4	+3	+2	+1	0	+1	+2	+3	+4
Temperature (rectal °C)	>41	39-40.9		38.5-38.9	36-38.4	34-35.9	32-33.9	30-31.9	<29.0
Mean arterial pressure (mm Hg)	≥160	130-159	110-129		70-109		50-69		≤49
Heart rate-ventricular response	≥180	140-179	110-139		70-109		55-69	40-54	≤39
Respiratory Rate	≥50	35-49		25-34	12-24	10-11	6-9		≤5
Oxygen*	≥500	350-499	200-349		<200 PO_2>70	$PO_2$61-70		$PO_2$55-60	PO_2<55
Arterial pH Serum HCO3-if no ABG's	≥7.7 ≥52	7.6-7.69 41-51.9		7.5-7.59 32-40.9	7.33-7.49 23-31.9		7.25-7.32 18-21.9	7.15-7.24 15-17.9	<7.15 <15
Serum sodium (mmol/l)	≥180	160-179	155-159	150-154	130-149		120-129	111-119	≤110
Serum potassium (mmol/l)	≥7	6-6.9		5.5-5.9	3.5-5.4	3-3.4	2.5-2.9		<2.5
Serum creatinine (μmol/l)	≥350	200-340	150-190		60-140		<60		
Haematocrit (%)	≥60		50-50.9	46-49.9	30-45.9		20-29.9		<20
White Blood cell count (x1000/mm3)	≥40		20-39.9	15-19.9	3-14.9		1-2.9		<1
Glasgow Coma Score	Score = 15 minus actual GCS								

B. Age Points

Age (yrs)	Points
≤44	0
45-54	2
55-64	3
65-74	5
≥75	6

C. Chronic Health Points

History	Points for elective surgery	Points for Emergency Surgery and non-operative patients
Liver	2	5
Cardiovascular	2	5
Respiratory	2	5
Renal	2	5
Immunocompromised	2	5

APACHE II Score: Sum of A+B+C

*Oxygen A-aDO_2 or PaO_2 (mmHg)

a) $FiO_2 \geq 0.5$ record A-aDO_2

b) $FiO_2 < 0.5$ record only PaO_2

Appendix I
Antibiotic Coding

Antibiotic Coding Chart

1. **Penicillins**

   Amoxicillin, Ampicillin, Aziocillin, Carbenicillin, Cloxacillin, Mezlocillin, Nafcillin, Penicillin, Piperacillin, Ticarcillin

2. **Aminoglycosides**

   Amikacin, Gentamicin, Kanamycin, Neomycin, Streptomycin, Tobramycin

3. **Macrolides**

   Azithromycin, Clarithromycin, Erythromycin, Troleandomycin

4. **Quinolones**

   Ciprofloxacin, Enoxacin, Norfloxacin, Ofloxacin, Levofloxacin

5. **Cephalosporins**

   Cefactor, Cefradroxil, Cefazolin, Cefixime, Cefoperazone, Cefotaxime, Cefotetan, Cefoxxitin, Ceftazidime, Cefriaxone, Cefuroxime, Cephalexin, Cephalothin, Loracarbet

6. **Antifungals**

   Amphotericin B, Fluconazole, Itraconazole

7. **Miscellaneous – Metronidazole**

8. **Miscellaneous – Vancomycin**

9. **Miscellaneous – Clindamycin**

Appendix J
Discharge Data

DISCHARGE DATA

Purpose:	**To collect patient information on discharge from ICU**

ICU ID Code _ _ _ **Study #** _ _ _ **Patient Initials (FML)** _ _ _

1. Date/ Time of ICU discharge/death: _ _ - _ _ _ _ - _ _ Time: _ _ _ _ _
2. Did this patient die during their Hospital stay? ☐$_1$ Yes ☐$_2$ No
   If yes answered to Question #2, complete Q 3 and 4. Otherwise proceed to Q 5
3. Date/time of death _ _ - _ _ _ _ - _ _ Time: _ _ _ _ _
4. Etiology of death
   (a) Immediate cause:_____ICD-9 _ _ _ - _ _
   (b) Antecedent cause:_____ICD-9 _ _ _ - _ _
   (c) Underlying cause: _____ICD-9 _ _ _ - _ _
   (d) Other significant conditions contributing to death:
   _____ICD-9 _ _ _ - _ _
   _____ICD-9 _ _ _ - _ _
5. Date of Hospital discharge _ _ - _ _ _ - _ _
6. Did the patient cross-over treatment at any time during the study? ☐$_1$ Yes ☐$_2$ No
7. Check one primary reason for ending study participation:
   ___Completed 7 days of therapy
   ___EN discontinued prior to 7 days of therapy
   a)__stopped
   b)__switched to TPN
   ___Family/patient decision to end
   ___Entry criteria not met
   ___Other_____